Der Weg ist das Ziel.

Department of Production Animal Medicine
Faculty of Veterinary Medicine
University of Helsinki, Finland

Luteinizing hormone and progesterone dynamics in the early pregnancy of the sow

Silke Marjatta Haen

Doctoral thesis, to be presented for public examination
with the permission of the Faculty of Veterinary Medicine
of the University of Helsinki, at Metsätalo, Hall 2,
on the 25th of September, 2020 at 12 o'clock.

Department of Production Animal Medicine
Faculty of Veterinary Medicine
University of Helsinki, Finland

Kliinisen Eläinlääketieteen Tohtoriohjelma (CVM)

Supervisors

Olli Peltoniemi
Professor, Department of Production Animal Medicine
University of Helsinki, Finland

Mari Heinonen
Professor, Department of Production Animal Medicine
University of Helsinki, Finland

Nicoline Soede
Professor, Department of Animal Sciences
Wageningen University & Research, the Netherlands

Pre-examiners

Ylva Brandt
Professor, Department of Clinical Sciences
Swedish University of Agricultural Sciences, Sweden

Christopher Grupen
Associate Professor of Reproductive Biology, Academic Advisor, Camden
Sydney School of Veterinary Science, Faculty of Science, Australia

Opponent

Pieter Langendijk
Adjunct Professor, Senior Reseacher
Murdoch University, Australia
Nutreco, Boxmeer, the Netherlands

Helsinki, 2020

The Faculty of Veterinary Medicine uses the Urkund system
(plagiarism recognition) to examine all doctoral dissertations.

ISBN 978-951-51-6471-1 (paperback)
ISBN 978-951-51-6472-8 (PDF)

Contents

1

Abstract

The hypothalamic-pituitary-gonadal axis is the endocrinological pathway of reproduction, where fertility is adjusted and fine-tuned with respect to intrinsic and extrinsic information. The pituitary gland receives the information that determines secretion of luteinizing hormone (LH) from the hypothalamus in the form of gonadotropin releasing hormone (GnRH), it is the frequency and amplitude of the GnRH pulses that regulate LH release. LH transports the information from the brain to the gonads, which feeds back to the hypothalamus and the pituitary gland in the form of steroids.

This thesis work focused on the orchestration of GnRH, LH and progesterone during the early pregnancy of the pig. Therefore, gonads refer to the ovary with their functional bodies, the corpora lutea (CLs) and their principal steroid, progesterone. CLs form from the follicles after ovulation and they are either maintained, and pregnancy continues, or they regress, enabling follicle growth and new ovulations and a new opportunity for reproduction. The corpus luteum is independent of pituitary LH support for the first eleven days after ovulation. Subsequently the maternal organism is informed of the presence of embryos and the need to maintain the CLs. Thus, dependency of the CLs on LH begins at the same time as the sow receives the positive signals that indicate pregnancy. Progesterone is the main hormone of pregnancy and its concentration is highest around the time of maternal recognition of pregnancy. LH supports the CLs, but it remains unclear for exactly how long during pregnancy the LH stimulus directly triggers secretion of progesterone.

The objective of this thesis work was to study the relationship between the two hormones, LH and progesterone, during early pregnancy in pigs. Firstly, we investigated the physiological release of LH and ovarian progesterone in inseminated gilts on Day 11 after oestrus, before the CLs are thought to be dependent on LH. Secondly, we looked at the pattern of progesterone release after the CLs had become dependent on LH. We studied the pattern of progesterone release from the ovary and were interested to ascertain if that pattern is related to the LH pattern. To explore a possible causal relationship between LH and progesterone further, a GnRH agonist model was used to nullify LH pulsatility around implantation of embryos. Thirdly, we were interested to know if parity,

sow age and maturity affect the relationship between the two hormones. In our studies, a catheter was inserted through the vena saphena lateralis, enabling blood sampling in the vena cava caudalis next to the venous drainage of the ovary and uterus. LH and progesterone concentration were assessed after frequent blood sampling for 8–12 hours on Day 11 after oestrus in inseminated gilts, and on Days 16 and 21 in pregnant gilts. A slow-release GnRH agonist was inserted into pregnant gilts on Day 11 and LH and progesterone were studied on Day 16 and Day 21. Additionally, a vena jugularis catheter was inserted into pregnant primiparous sows and progesterone concentrations in local (vena cava caudalis) and peripheral (vena jugularis) blood samples were compared on Day 14.

In gilts that were inseminated and classified post mortem whether they were pregnant or not, the progesterone release pattern measured in the vena cava caudalis had similar basal and mean progesterone concentrations, and frequency and amplitude of pulses on Day 11.

On Day 11 and Day 21 of pregnancy, there was no relation between LH pulsatility and progesterone pulsatility. In gilts that were treated with a slow-release GnRH agonist on Day 11, LH pulsatility ceased but progesterone release remained pulsatile on Day 16 and Day 21 of pregnancy. On Day 21 we found that LH pulsatile release was active when progesterone release was basal. In primiparous sows, a progesterone pulse followed 60.8% of LH pulses within one hour. Mean progesterone concentration was approximately twice as high in the vena cava caudalis than in the vena jugularis in primiparous sows and differed significantly before and after feeding in the vena jugularis. GnRH-agonist-treated gilts had an elevated progesterone concentration on Day 21 compared with the control gilts. Progesterone concentration declined from Day 11 via Day 16 to Day 21 in gilts. LH pulse amplitude declined during those days, but no decline in mean or basal LH concentration or LH pulse frequency was recorded. LH pulsatility ceased on Day 16 and Day 21 in gilts treated with the GnRH agonist. LH release was synchronized in such a way that gilts under the same management conditions exhibited LH pulses around the same time during twelve hours, indicating a synchronized rhythm of secretion.

We observed that progesterone release of CLs is similar in non-pregnant gilts and pregnant gilts on Day 11, before maternal recognition of pregnancy. During early pregnancy in gilts the pulsatile progesterone release of CLs on Days 11 and 16 is not responsive to – and on Days 16 and 21 it is not dependent on – LH pulsatile secretion. On Day 21 there is an alternate pattern of activity in pulsatile release of LH

and progesterone in individual gilts. In primiparous sows, we found a temporal relationship between LH pulses and progesterone pulses already on Day 14 of pregnancy. We conclude that the progesterone release of the CLs functions without LH pulsatile stimulus from Day 11 to Day 21, but associations of these hormones at an individual level are evident.

2

General introduction

Female sexual endocrinology across species is, for me, one of the most fascinating subjects in the life sciences. Reproduction is a simple process on the one hand and yet so complex on the other hand. Yet, when searching for solutions to problems related to reproduction in my profession, I came to understand that this field still attracts considerable investigation. Although high pregnancy rate and litter size are crucial for meat production animals, information on these issues is lacking. When working in piggeries, I did not understand why dealing with reproduction of gilts and sows was time consuming, expensive and often frustrating, and I wanted to know more about reproduction. With this thesis work, I aimed to explore important aspects of porcine reproduction.
The studies of Peltoniemi et al. (1999), Tast et al. (2001) and Virolainen et al. (2004) demonstrated that although we have succeeded in domesticating the wild boar (sus scrofa scrofa) and using it for our purposes, we have not given due attention to the origin of modern sows. We expect from a former distinctive seasonal breeder that the pig breeds year round. On my literature review journey I found that at the endocrinological level this expectation is challenged. Where Peltoniemi et al. (1999) surveyed the impact of season on pig reproduction, Tast et al. (2001) looked at the relationship of light (melatonin) and hormonal patterns with regard to reproduction. Virolainen et al. (2004) investigated whether feeding could overcome some of the negative effects that season has on reproduction, and fortuitously determined a possible direct relationship between two hormones (LH and progesterone) important for reproduction. They researched in vivo developments at the beginning of the fourth week of pregnancy in gilts. Virolainen et al. (2005) measured progesterone near the ovary.
We therefore opted to research the dynamics of local release of progesterone during early pregnancy and expand on current knowledge of the underlying processes.

3

Review of literature

3.1 Overview of early pregnancy

The duration of pregnancy in the sow is 114 to 115 days. The first 35 days of gestation are defined as early pregnancy and referred to as the embryonic phase of pregnancy (Senger, 2012). The embryonic phase is generally characterized by the early stages of growth and differentiation. More specifically, that means cleavage, the laying down of fundamental tissues, and the formation of primitive organs and organ systems. Subsequently the foetal phase of pregnancy occurs and the developed embryo is addressed as the foetus.

3.1.1 Embryonic development

After ovulation and subsequent fertilization in the fallopian tubes, the fertilized ova reach the uterus, change their morphology and signal the dam of their existence (Geisert et al., 1982). With their signalling they ensure sustained progesterone release from the ovaries, which is essential to maintain pregnancy in sows (Ziecik et al., 2018). Additional to progesterone at the end of a hormonal communication cascade, several other hormones represent components of the cascade. The superior hormone is the hypothalamic gonadotropin-releasing hormone (GnRH), which regulates the release of luteinizing hormone (LH) from the pituitary gland (Brussow et al., 2007). LH can be seen as the connective link between brain and reproductive organs and supports the function of the temporary gonadal glands, the corpora lutea (CLs). They function without support of LH until the 12th day of pregnancy (Anderson et al., 1967), after which LH is required (Kraeling et al., 1974) otherwise the CL will degenerate. Progesterone is the main hormone that maintains pregnancy and is built of cholesterol in the CL and influences the oviductal and endometrial development. Progesterone induces secretion of histotrophes from the endometrium that are required by the growing embryos and progesterone is also the raw substance for further metabolization by the embryos into oestradiol (Geisert et al., 1982). Embryonic oestradiol stimulates a cascade of events at the end of which the CLs are rescued (at about Day 12 of pregnancy) and the pregnancy

is maintained (van der Meulen et al., 1988). This one signal alone is not enough to maintain pregnancy, but a second prolonged oestrogenic signal is required to maintain luteal function and thereby guarantee the survival of the embryos. This second signal can only appear if there is sufficient progesterone (Pusateri et al., 1996a; Pusateri et al., 1996b). The timing of the second oestrogenic signal differs among studies, but in pregnant gilts oestrogens in uterine flushings start to increase in concentration for the second time by Day 15 (Stone and Seamark, 1985). A minimal number of 4 embryos have to be present in the uterus in order to establish and maintain pregnancy (Polge et al., 1966). Before Day 12 presence of embryos in both horns whereas after Day 12 occupation of only one horn is required (Dhindsa and Dziuk 1968).

3.1.2 Interruption of pregnancy

These communication processes between the embryo and the dam and between the brain and the ovary of the dam are very complex. The correct timing of progesterone release and the required level of progesterone seem to be essential for synchronized embryonic development (Pope, 1988), which is fundamental for a successful pregnancy. The early stage of pregnancy is a critical time across species during which the entire cascade can be disturbed and lead to the disruption and termination of the pregnancy. In Holstein-Friesian dairy cows, up to 45% of the pregnancy losses occur during the embryonic phase (Santos et al., 2004; Walsh et al., 2011). In women, it has been reported that miscarriage is the most common complication of early pregnancy and that about 80% of miscarriages occur in the first 12 weeks of pregnancy (Bienstock et al., 2015).
Cattle and humans usually carry only one conceptus and its death results in the interruption of pregnancy. The pig has multiple ovulations and hence several conceptuses. Prenatal mortality has been reported to range between 20% and 46% by term (Pope, 1994) or 10–15% (Ostrup et al., 2008), with the majority of the losses occurring between Day 12 to Day 18 of gestation (Stroband et al., 1990). Due to the different speeds of development, the embryos reach the uterus at different developmental stages, resulting in unfavourable uterine conditions and consequent death for the least developed embryos (Pope, 1994). The more developed embryos survive and pregnancy continues. Synchronized embryonal and uterine development depends on progesterone and is

the premise for successful embryonal attachment (Geisert et al., 1982). Complete termination of pregnancy, however, may also be observed in sows and might be due to viral, bacterial or parasitical infections, poor management conditions or early selection for genetic defects (Ramirez et al., 2019).

3.1.3 Seasonality of breeding in the pig

From an endocrinological perspective, the European wild boar as a strict short-day seasonal breeder may set an example of seasonality for breeding in the modern prolific sow. The seasonal breeding pattern of the European wild boar enables birth of the litter during the time of the year favourable to raise offspring. Reduced fertility can be manifested in delayed puberty (Paterson and Pearce, 1989), prolonged weaning to oestrus interval (Peltoniemi et al., 1999) and seasonal effects on boar fertility (Claus and Weiler, 1985). A reduced farrowing rate (Love, 1981) is thought to be consequential for early disruption of pregnancy (EDP) and is manifested in a delayed return to oestrus between Days 25 and 35 after mating (Love, 1981). In sows experiencing EDP, fertilization and viable embryos occur apparently normally, but the pregnancy is interrupted and the whole litter is lost (Love et al., 1981). The typical oestrus interval in delayed returns is 25–35 days, implying that the second embryonic signal is inadequate or absent (van der Meulen et al., 1988). Reduced or even barren fertility during summer and autumn ensures reduced farrowing rate or maybe no offspring at all during late autumn and winter. EDP is a global problem, in the Northern hemisphere EDP occurs from August to October and is mainly found in gilts and primiparous sows. A 5–10% reduction in the farrowing rate following matings in these months of the year (Peltoniemi et al., 1999) was reported and this reduction happens partly because of EDP (Tast et al., 2002). In conclusion, there may be many reasons for interruption of pregnancy, but the discontinuation of progesterone release in all cases results in a discontinuation of pregnancy.

3.1.4 Progesterone dynamics in systemic vs. local circulation

Progesterone and LH profiles throughout the cycle and pregnancy of the pig are well described in the literature. The majority of the studies measured progesterone in serum from peripheral blood, e.g. from the vena jugularis, meaning that the blood had flowed through the liver and been metabolized (Prime and Symonds, 1993) (Figure 1). Consequently, the vast majority of studies do not represent progesterone in the manner in which it is released by the CL (Pharazyn et al., 1991). The progesterone measured in the periphery does not always show a pulsatile pattern and the concentration is lower after having been metabolized (Virolainen et al., 2005). Therefore, questions regarding changes in progesterone release can only be answered if progesterone is measured before it reaches the liver.

Apparently, countercurrent transfer from the ovarian veins into the uterine arterial blood is possible between the venous and the arterial systems along the mesometrium. Both utero-ovarian veins drain into the vena cava caudalis and thus blood is transported from the genital tract via the liver to the heart (Stefanczyk-Krzymowska et al., 1998) (Figure 1). Blood from the vena cava caudalis can be drawn with the help of a vena cava caudalis catheter via the vena saphena lateralis (Benoit and Dailey, 1991). Virolainen et al. (2005) discovered a considerable difference between progesterone concentration in the blood, which was sampled from the vena cava caudalis, and the progesterone concentration in the blood sampled from the vena jugularis. They suspected that progesterone pulses, or a rise in progesterone, respectively, followed after LH pulses and that there was an indication of an episodic pattern of progesterone production on Day 22 of pregnancy. To illustrate the connection between LH and progesterone during early pregnancy in the sow, the secretion of hypophysial LH, the anatomic backgrounds, the function of the CL and the release of progesterone will be reviewed here. Whenever possible, the focus is on results of studies exploring early pregnancy in the sow.

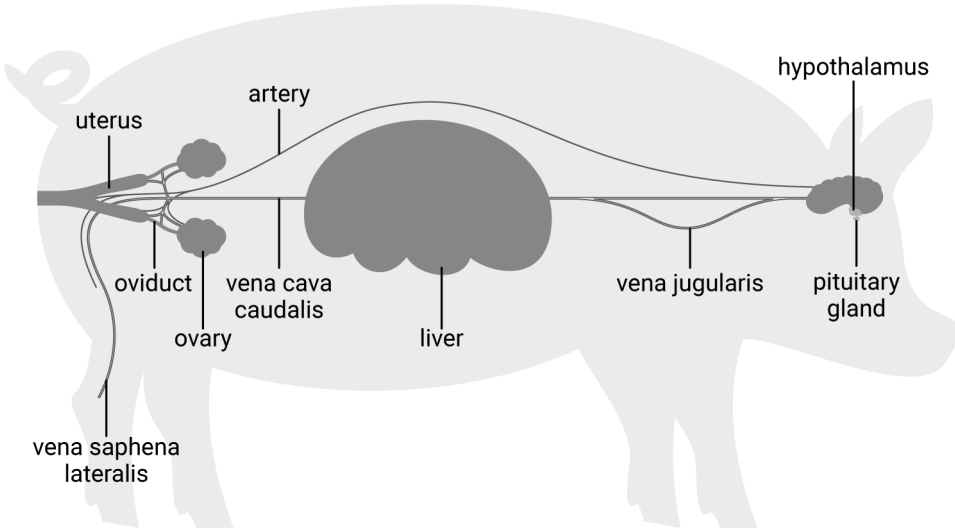


Figure 1 Schematic drawing of a sow showing the main organs referred to in this thesis.

3.2 Hypothalamus and hypophysis

3.2.1 GnRH and LH

Following an environmental or physiological stimulus (Constantin, 2017) from the central nervous system, the endocrine neurons in the hypothalamus produce GnRH (Marques et al., 2018). The hypothalamus releases GnRH from its surge centre in highly frequent and high amplitude pulses over a short period (hours), whereas GnRH is released from the hypothalamic tonic centre in low frequency, low amplitude episodes (Senger, 2012). GnRH secretion is pulsatile and GnRH is transported via the hypothalamo-hypophyseal portal system into the anterior lobe of the pituitary gland, where it stimulates the secretion of LH (Leshin et al., 1992) and follicle stimulating hormone (FSH) (Ye et al., 2013). The secretory activity of the pituitary gland is modulated by the amplitude and frequency of GnRH pulses rather than the constant concentration of the hormone (Leshin et al., 1992). LH is a glycoprotein produced by the gonadotropic cells in the anterior pituitary gland that is released in a pulsatile manner (Williams, 1989) and transported via the blood to the ovary, where it stimulates follicle development, triggers ovulation, supports the transformation of the follicle into the CL and maintains the CL throughout pregnancy (Anderson, 1967; Kraeling et al., 1974).

The regulation of the GnRH pulse generator is still a subject of research. In small ruminants and mice, a group of cells within the arcuate nucleus of the hypothalamus (ARC), termed kisspeptin- neurokinin B-dynorphin (KNDy) neurons, coexpress kisspeptin, neurokinin B (NKB) and dynorphin (Navarro et al., 2009; Wakabayashi et al., 2010). Neurokinin B is thought to act through an autoregulatory mechanism to induce the release of kisspeptin, which acts directly on the GnRH neuronal network to stimulate a pulsatile release of GnRH and subsequently LH. The KNDy neurons are therefore positioned to relay information about interoceptive signals, such as gonadal steroids and metabolic hormones, to modulate the frequency and amplitude of GnRH pulses. Mutations resulting in the loss of function of kisspeptin and NKB or their respective receptors lead to hypogonadotropic hypogonadism and failure to attain puberty in pigs (Sonstegard et al., 2016). In prepubertal gilts, kisspeptin stimulates gonadotropin secretion (Clay et al., 2008); in the mouse and in the rat this stimulation happens through direct receptor action on GnRH neurons (Messager et al., 2005 (mouse) and Irwig et al., 2004 (rat)). Expression of kisspeptin and neurokinin B in pigs is altered under different conditions

of gonadal feedback (Tomikawa et al., 2009). Moreover, hypothalamic expression of kisspeptin was correlated with the number of LH pulses in the pig, and amplitudes of LH pulses were possibly regulated by NKB in the gilts (Foote et al., 2018).
Internal factors, such as the gonadal feedback mechanism, and external factors, such as photoperiod, nutrition and metabolic status, exert their effects on the reproductive pattern through the modulation of GnRH secretion by the hypothalamus.

3.2.1.1 Internal modulators of GnRH action

Internal ovarian modulators of GnRH release are oestradiol and progesterone. Oestradiol yields a positive feedback on the hypothalamus, increasing the frequency of the GnRH pulses. Above a certain threshold level of oestradiol the hypothalamus responds with a surge of GnRH that results in the pre-ovulatory LH surge (Knox et al., 1991). Ovulation takes place on average 27 to 33 hours after the peak of the LH surge, which occurs 41 to 47 hours after the onset of the oestradiol surge (Soede et al., 1994). After ovulation, progesterone begins to feed back to the hypothalamus. Progesterone inhibits GnRH neurons and high progesterone concentrations reduce the frequency of the basal episodic secretion of GnRH. The frequency of the GnRH pulse generator is reduced so that LH pulses occur at three to four hour intervals (Langendijk et al., 2007). If there is no gonadal feedback, for example after castration or after the menopause in humans, LH is elevated (Han et al., 2017).

3.2.1.2 External modulators of GnRH action

Photoperiod

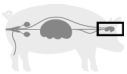
Seasonal changes in photoperiod, both the length and intensity of light, play an important role in GnRH secretion (Smith and Almond, 1991). The stimulation of the retina (photoreception) is transmitted via nerve tracts to the pinealocytes in the pineal gland (Ebling and Hastings, 1992). Pinealocytes produce melatonin, which in turn is a stimulator for GnRH secretion. Porcine serum melatonin concentration rises with the onset

of darkness (Tast et al., 2002). Consequently, pregnant gilts' LH pulse frequency and amplitude are high in a season with a shorter photoperiod (January and February in the Northern Hemisphere). In contrast, LH pulse frequency and amplitude appear to be lower in a season with a long photoperiod, as in August and September in the Northern Hemisphere during mid-gestation (Smith and Almond, 1991) and during early pregnancy (Day 14) (Peltoniemi et al., 1997). LH secretion probably represents the response of the pig to the photoperiod; during the course of evolution, the pig has adjusted to the environment to enable the offspring not to be born in winter, when the chances of survival are low (Peltoniemi and Virolainen, 2006).

Nutrient factors

Influence of feed intake on LH secretion is not yet completely understood and is currently an important subject of research. Progesterone concentrations are especially high during the second week of pregnancy. This high progesterone activity presents the hypothalamus with a strong negative feedback effect that suppresses LH production and might counter impacts of nutrition on LH secretion.
Peltoniemi et al. (1997) reported no effect of a moderate feed restriction on LH frequency and mean concentration during early pregnancy (Peltoniemi et al., 1997). In addition, acute fasting during Days 10 and 11 of early pregnancy in gilts did not have an immediate effect on LH secretion (Langendijk et al., 2017). On the other hand, high energy feed intake from Day 0 to Day 14 after insemination led to significantly higher LH amplitude on Day 14 (Peltoniemi et al., 1997) compared with groups on lower energy intake. Furthermore, feed reduction during the first two weeks of gestation to 50% of ad libitum reduced LH pulse frequency compared with the ad libitum feed group (Peltoniemi et al., 1997). Zhou et al. (2014) found that severe nutrient restriction (1 kg food/day for over 90 days) downregulated gene-expression of gonadotropin releasing hormone and that depressed GnRH secretion was the main cause for the inhibition of gonadal function and reproductive failure in nutrient-restricted gilts. LH pulse amplitude of ovariectomized prepubertal gilts was higher in feed-restricted gilts (feed restriction for 11 days) compared with the fully fed (three times maintenance) group (Foote et al., 2018). It seems therefore that not only the amount of feed and its energy content but also the source of energy and the duration of feed restriction act on LH secretion pattern (Prunier and Quesnel, 2000). The effects of feed

characteristics on LH are due to changes in circulating concentrations of metabolic fuels (glucose, free fatty acids (FFA)) and metabolic hormones such as insulin, leptin and insulin-like growth factor-1 (IGF-1), which feed back to the hypothalamus and thus link metabolic status to the reproductive neuroendocrine axis (Foote et al., 2018). Metabolic state, specifically energy balance, is a potent regulator of leptin secretion and gene expression (Whisnant and Harrell, 2002) (Figure 2). KNDy neurons appear to be targets for metabolic hormones such as leptin (Smith et al., 2006, Quennell et al., 2011) and IGF-1 (Hiney et al., 2009) in rodents, suggesting they also play a key role in integrating metabolism and reproduction. Levels of insulin and corresponding IGF-1 are related to LH secretion during the weaning to oestrus interval (Wientjes et al., 2012). The role of leptin is to stimulate secretion of GnRH from the porcine hypothalamus (Figure 2) and it can also stimulate release of LH from the anterior pituitary gland of the pig (Siawrys and Gajewska, 2017; Barb et al., 2005) (Figure 2). However, associations between leptin and reproduction are not always found. For example, feed restriction for seven days suppressed both serum leptin concentrations and pulsatile LH secretion in mature ovariectomized gilts (Barb et al., 2001), but although 24 h fasting led to a decrease in pulsatile leptin secretion, it did not change LH secretion (Barb et al., 2001). Thus, it is not sufficiently clear when leptin affects pulsatile secretion of GnRH and LH in gilts (Barb et al., 2008), let alone if leptin is associated with the HPG axis during early pregnancy in the pig.



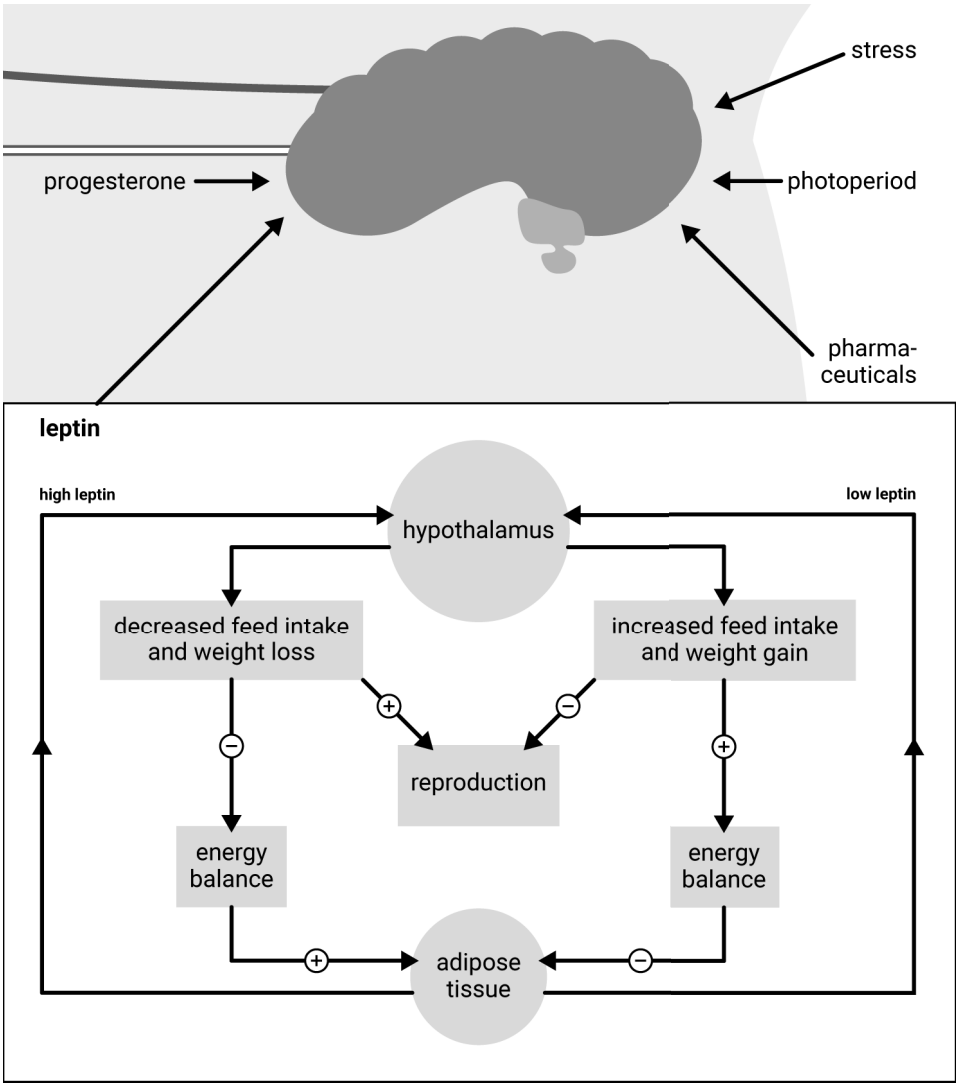


Figure 2 The adipose tissue secretes leptin in response to changes in energy balance. Leptin acts on the hypothalamus to control food intake, reproduction and adipocyte function. Positive energy balance increases blood leptin concentrations. Negative energy balance decreases blood leptin concentrations (modified from Barb et al., 2005).

Stress

Animal reproduction is sensitive to stress (Dobson and Smith, 2000). Stress in pigs has been mainly studied mimicking stress-induced reaction by injections of ACTH (Razdan et al., 2002) or cortisol (Turner et al., 1999). Additionally, research has focused on the influence of stress on embryonic survival, especially addressing group-housed sows. A review by Spoolder et al. (2009) concentrated on the literature to investigate whether stress affects embryonic mortality in group-housed sows, but different studies seem to provide contradictory results. Munsterhjelm et al. (2008) reported a tendency towards more early loss of embryos and an increased rate of repeat breeding after a prolonged oestrus-to-oestrus interval, whereas Einarsson et al. (2014) found that reproductive performance was not impaired in group-housed sows compared with conventionally kept sows. Soede et al. (2007) demonstrated that repeated acute stress did not affect reproductive processes during early pregnancy. LH responses to stress vary in response to factors such as type of stressor, duration, intensity and individual variation among pigs (Dobson and Smith, 2000; Einarsson et al., 2008). LH release was inhibited by sustained (16 days) elevation of cortisol in ovariectomized pigs and in intact gilts sustained (about 20 days) elevation of cortisol impaired the preovulatory LH surge (Turner et al., 1999 a and b). On the other hand, ACTH administration for 48 hours during standing oestrus did not significantly alter the LH concentrations in sows compared with the control group (Brandt et al., 2007). However, the interval of the LH peak to ovulation was prolonged by the ACTH administration (Brandt et al., 2007 a and b). Moreover, in the experiment of Zhu et al. (2016), ACTH treatment for seven days resulted in elevated cortisol concentrations, which reduced steroidogenic hormone concentrations and LH receptor expression (Zhu et al., 2016). These results indicate that stress may act directly on the HPG axis of pigs.

Pharmaceutical modulation

Several pharmaceuticals that mimic or remove GnRH function and subsequent LH release (van Loenen et al., 2002) have been studied in the pig (Figure 3).

GnRH agonist

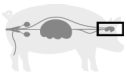
GnRH agonists are modelled on the hypothalamic neurohormone GnRH and interact with the GnRH receptor (Tzoupis et al., 2019). The interaction leads to an initial flare response and the continued stimulation with the GnRH agonists desensitizes the pituitary gland to GnRH by initiating a downregulation of the GnRH receptor. This pituitary desensitization reduces the secretion of LH and suppresses gonadal steroid hormone production (Peltoniemi et al., 1995) (Figure 3). GnRH agonists are either short acting or long acting devices. In human medicine, they are used for a variety of indications, including fertility medicine and the lowering of sex hormone levels, for example in prostate cancer (Magon, 2011). In veterinary medicine, suppression of testosterone and related sexual activity in male dogs (Junaidi et al., 2007) (long acting) or induction of ovulation and support of the developing CL in cows (Freick et al., 2014) (short acting) has been achieved with GnRH agonists. The application of a GnRH agonist during early pregnancy in the pig has led to different results, depending on the device. A short acting GnRH agonist injected on Day 12 after insemination first induced an initial LH rise and then an elevated basal concentration on Days 13, 15 and 17, but did not influence mean LH concentration and pulse frequency or amplitude on the same days in pregnant gilts (Brussow et al., 2011). However, a chronic application (long acting) of a GnRH agonist on Day 14 to Day 21 of pregnancy (Peltoniemi et al., 1995) resulted, after a short period of increased LH secretion, in cessation of LH secretion for 20–22 days.

GnRH antagonist

Another possibility to model GnRH production is the use of a GnRH antagonist. GnRH antagonists compete with the GnRH molecule, they block the GnRH receptor and as a consequence the production of LH and FSH. GnRH antagonists are used in human medicine, for example, for ovarian stimulation in assisted reproduction protocols (Brady et al., 2014). GnRH antagonists start to act immediately and rapidly block secretion of LH without any initial surge for about 2.7 days, as reported in Virolainen et al. (2004) between Days 14 and 19 of pregnancy in sows (Figure 3).

Immunization against GnRH

Another alternative to block GnRH activity is immunization against GnRH. The GnRH molecule binds to an antibody and is neutralized after which it no longer fits into the receptor (Figure 3). Commercial GnRH vaccines have been designed for domestic animals and tested in pigs (Karakonji et al., 2015), cattle (Balet et al., 2014) and horses (Janett et al., 2009). Tast et al. (2000) immunized gilts passively 12 days after mating and LH pulsatility ceased completely the day after treatment.



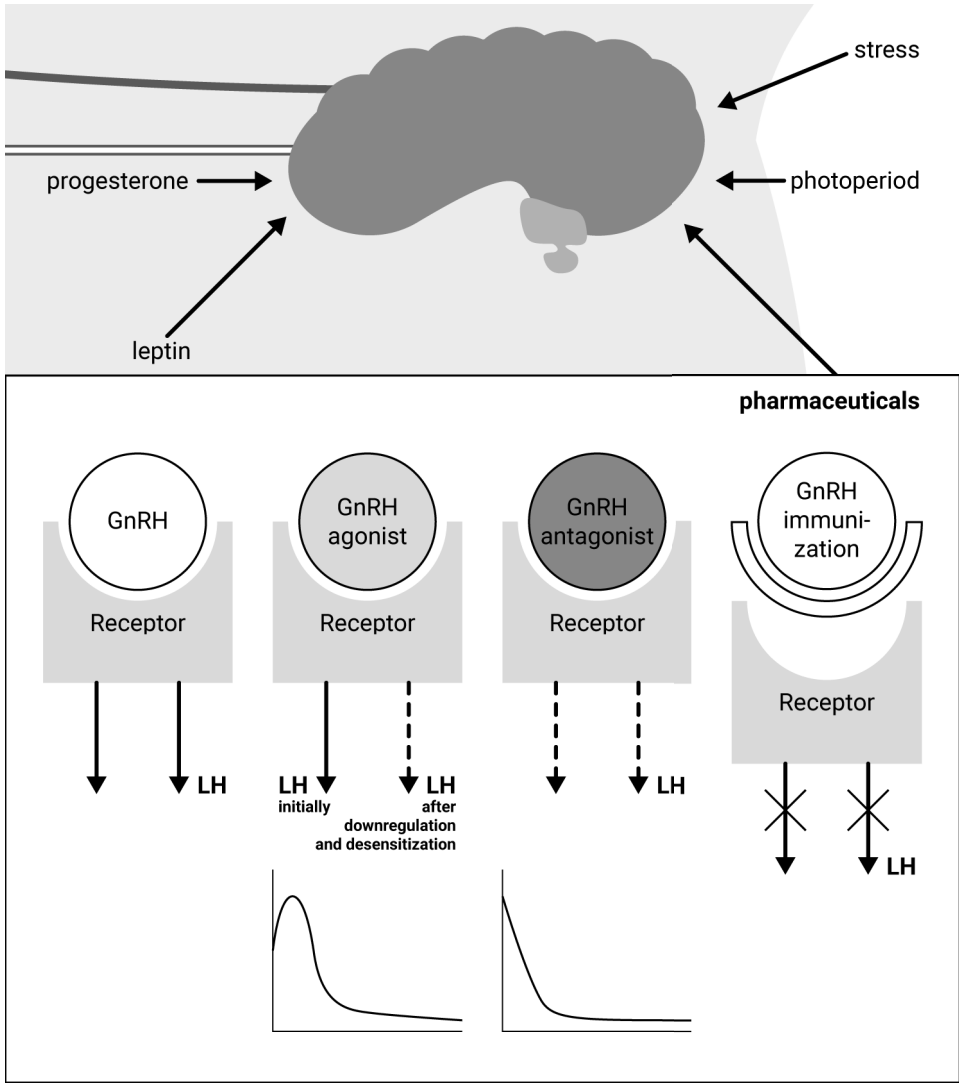


Figure 3 Mechanism of action of the GnRH molecule, GnRH agonist, GnRH antagonist and immunization against GnRH with regard to LH release from the pituitary gland. GnRH binds to the receptor and LH is released. The GnRH agonist binds to the receptor and initiates LH release; the continued stimulation leads to a downregulation of receptors and to a reduction of LH production. The GnRH antagonist blocks the receptor for GnRH and the LH production is stopped. Immunization against GnRH leads to presence of GnRH antibodies, which bind the GnRH molecule and consequently it ceases to fit into the receptor; finally, no information is given to the pituitary gland to produce LH. Modified from Peltoniemi et al. (1995), Tast et al. (2000) and Virolainen et al. (2004).

3.3 Corpus luteum

3.3.1 Formation of the CL

The CL might be regarded as a time-limited gland, functioning to support and maintain pregnancy. The CL forms from cells of the ovarian follicle wall during ovulation (Baerwald et al., 2005) and consists of different cell populations, such as small luteal cells, large luteal cells, fibroblasts, capillary endothelial cells and pericytes (Farin et al., 1986). One CL forms out of each ovulated follicle. It seems that non-pregnant pigs contain a homogenous population of CL such that an individual CL reflects the characteristics of its cohorts (Ottobre et al., 1984). Whether that is also true for the CL of pregnancy remains unclear.
Histological evaluation of the CL revealed fresh bleeding into the central cavity following follicle rupture (Corner, 1956) and therefore the newly formed CL is referred to as the 'corpus hemorrhagicum'. The follicular wall infolds after ovulation and facilitates the migration of fibroblasts, endothelial cells and theca interna cells into the central regions of the developing corpora lutea. During the process of luteinization, changes appear in the cellular organelles that participate in steroid production. These changes include an increase in the smooth endoplasmic reticulum, increased size of the Golgi apparatus and an increased number and complexity of mitochondria (McClellan et al., 1975). Growth of the CL is mainly the result of tissue growth and cellular proliferation (Murphy et al., 2001) and is associated with an increase in luteal blood flow and serum progesterone concentrations (Murphy et al., 2001; Langendijk and Peltoniemi, 2013). In the pig, the CL area increases from approximately 0.35 cm2 at 24 hours after mating to 1.0 cm2 six days after mating (Tast et al., 2002) and the porcine CL reaches its full size between 10 and 12 days after ovulation (Langendijk and Peltoniemi, 2013). The CL growth in domestic animals is believed to be the result of an increase in the size of large luteal cells (Zheng et al., 1994), and an increase in the number of small luteal cells, fibroblasts and endothelial cells (Reynolds and Redmer, 1998).
Due to the proliferation of endothelial cells, the corpus luteum has an extensive capillary network (Redmer et al., 1996). The mature CL is highly vascular and receives one of the greatest rates of blood flow per unit of tissue mass, of any organ (Redmer et al., 1996). Due to the extensive capillary network of the mature CL, the majority of steroidogenic cells are adjacent to one or more capillaries (Zheng et al., 1994).
The CL either regresses at the end of the luteal phase of the oestrus

cycle and the remnants of the CL are then referred to as the corpus albicans, or the CL are rescued and transformed into the CL graviditatis (Senger, 2012). It is believed that luteal cells originate from the theca and granulosa cells following breakdown of the basal lamina immediately prior to follicle rupture (Sanders et al., 1996). Two subtypes of luteal cells are evident. Large luteal cells (LLC) form out of the granulosa cells of the ovulated follicle and they are sometimes referred to as granulosa lutein cells, whereas small luteal cells (SLC) form out of follicular theca cells, and may be referred to as theca-lutein cells (Lemon and Mauleon, 1982). However, some studies also report the differentiation of small luteal cells into large luteal cells (Cran, 1983; Fritz and Fitz, 1991), which together are the only steroidogenic cells in the corpora lutea of the pig (Lemon and Loir, 1977).

3.3.2 Peptides and hormones of the CL

Luteal cells have specific receptors for LH on their plasma membrane. After LH-binding, the LH-receptor-complex activates via the cAMP second messenger the transportation of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, supported by steroidogenic acute regulatory protein, StAR. The favoured effective and usable form of cholesterol seems to be low-density lipoproteins (Richards et al., 1994). In the mitochondrion the P450 enzyme complex for cholesterol side-chain cleavage (P450scc) converts cholesterol to pregnenolone, which is a precursor of progesterone. After leaving the mitochondria, pregnenolone is converted enzymatically to progesterone.

Both SLC and LLC produce progesterone. The two different cell types are differently stimulated by LH in vitro. SLC retrieved at Day 8 to Day 9 of the oestrus cycle seem to be more responsive to LH than LLC (Richards et al., 1994). LH enhanced progesterone production in SLC but had no or minimal stimulatory effect on LLC (Tekepetey and Armstrong, 1991: Day 12 to Day 14 of oestrus cycle). Wuttke et al. (2001) postulated that the small luteal cells in humans are responsible for the pulsatile progesterone release whereas large luteal cells might be the basal progesterone pacemakers.

In addition to progesterone, the porcine CL produces oestradiol, as demonstrated from Day 5 to Day 7 of the oestrus cycle in vivo (Jarry et al., 1990) and on Days 10–12 and 14–16 of pregnancy in vitro (Kurzynska

et al., 2014) and the luteal fibroblasts produce prostaglandins (PGs) (Wuttke et al., 1998). During the early luteal phase luteotropic PGs, for example PGE2, are synthesized, but in the mid-late luteal phase the production of PGF2alpha increases, resulting in an increased intraluteal PGF2alpha: PGE ratio (Waclawik et al., 2008) with a maximum at Day 13–14 of oestrus (Chang et al., 2017).

The large luteal cells also produce the neurohypophysial hormone oxytocin. The stimulus for oxytocin secretion is not clear. In sheep, endometrial PGF2alpha triggered luteal oxytocin release (Flint and Sheldrick, 1982). In bovine CL, oxytocin secretion is stimulated by catecholamines transported to the ovary by nerves (Luck and Jungclas, 1988). Jarry et al. (1990) revealed a significant correlation between the episodic release of oxytocin and pulses of progesterone intraluteally in Gottinger miniature pigs 5–7 days after oestrus. There were indications of a simultaneous release of oxytocin from the CL of both ovaries (Jarry et al., 1990).

In vivo intraluteally administered oxytocin stimulated the release of oestradiol and progesterone in a dose-dependent manner and oestradiol infusions into the CL stimulated progesterone release (Jarry et al., 1990) from Day 5 to Day 7 of the oestrus cycle (Figure 4). Oxytocin and PGF2alpha inhibited progesterone production, but at the same time they induced a strong increase of oestradiol secretion in small and large luteal cell cultures in vitro from young, 4 to 6 day old, CL (Pitzel et al., 1993). Oestradiol exerted a powerful stimulatory effect on progesterone secretion (Pitzel et al., 1993). Therefore, in the pig's young CL, oxytocin and PGF2alpha seem to mediate a luteotropic effect via oestradiol on progesterone secretion (Figure 4). In contrast, in luteal cells from old cyclic CL close to luteolysis (Day 12–14), oxytocin and PGF2alpha had no or only a moderately stimulatory effect on luteal oestradiol secretion, but now exerted a strong inhibitory effect on progesterone secretion (Wuttke et al., 1998) (Figure 6).

As described, the processes within the CL are very complex and are still a topic of research. The studies mentioned above on oestradiol, oxytocin, PG and progesterone indicate that the CL is responsive to its own hormones. Indeed, oestradiol receptor mRNA and localization were demonstrated in the pig CL at different days throughout pregnancy (Knapczyk et al., 2008). Progesterone receptor mRNA was up-regulated in vitro by LH in cultured pig granulosa cells (Iwai et al., 1991) and progesterone receptors have been demonstrated to be present in large luteal cells (Wuttke et al., 1998). The concentration of progesterone at Days 12, 18, 25 and 35 of pregnancy in the blood of the ovarian

artery was higher than in the systemic blood reaching the initial part of the ovarian artery. This finding led to the suggestion that this high progesterone concentration must affect the secretory function of the ovary (Stefanczyk-Krzymowska et al., 2004). The possibility of an autocrine and/or paracrine stimulation led to the idea of an intraluteal circuit in the pig (Wuttke et al., 1998) (Figure 4). This intraluteal circuit, also referred to as the intraluteal pulse generator, is triggered and maintained by regularly occurring LH pulses. High-affinity LH receptors have been identified in the porcine CL throughout the luteal phase (Ziecik et al., 1980; Rao and Edgerton, 1984). It was demonstrated that LH receptor density was decreased until the sixth day after ovulation during early CL development (Meduri et al., 1996). It might be that LH has a physiological role after all, even if only supplemental in regulating the steroidogenic function of the porcine CL during the oestrus cycle.

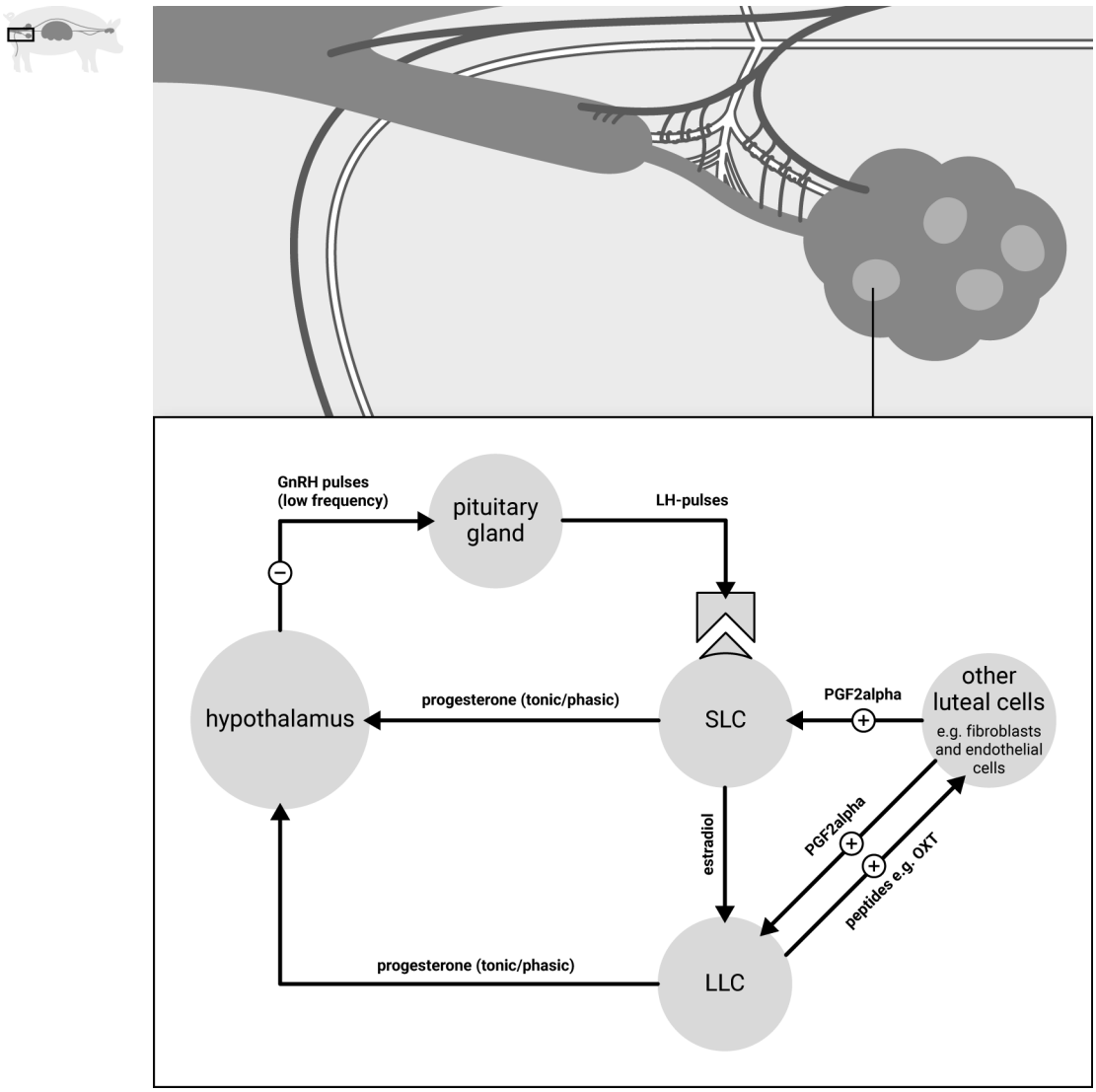


Figure 4 Proposed neuroendocrine and intraluteal oestrogenic luteotropic circuit regulating mid- luteal progesterone secretion. Combined tonic and phasic progesterone secretion stimulate beta- endorphin release (not shown in figure), which causes the hypothalamic pulse generator to function at a low frequency. This results in infrequent luteinizing hormone (LH) pulses at high amplitude. The LH pulses stimulate oestradiol and progesterone release from small luteal cells (SLC). The increased oestradiol stimulates further oestradiol secretion from large luteal cells (LLC) and the release of peptides, for example, oxytocin (OXT). The peptides stimulate fibroblasts

to release prostaglandin F2alpha (PGF2alpha) that stimulates further oestradiol release from SLC and LLC, which in turn stimulates more progesterone release. The direct inhibitory effect of PGF2alpha on progesterone release from both cell types is overridden by oestradiol, in that the oestradiol stimulation of progesterone is stronger than the PGF2alpha-inhibition of progesterone release.

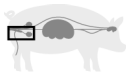
(Modified after Wuttke et al., 1998.)

3.3.3 CL dependency on LH

Hypophysectomy, as done by Anderson et al. (1967), administration of LH antiserum (Spies et al., 1967) or decreased levels of LH by progesterone administration (Woody et al., 1967) demonstrated that the CL can work without LH support up to Day 12 of the oestrus cycle after the preovulatory surge of LH. However, it was demonstrated that passive immunization of the non-pregnant gilt with porcine anti-LH antibodies decreased progesterone concentrations already at Day 8 of the oestrus cycle (Szafranska and Ziecik, 1989). In vitro progesterone secretion was induced by LH in slices of non-pregnant gilts' CLs collected on Days 10–12 (Przygrodzka et al., 2014). Thus, it seems that the LH-independent CL is sensitive to LH stimulus regarding progesterone release.
After Day 12 of pregnancy, the use of a GnRH-agonist (Peltoniemi et al., 1995), immunization against GnRH (Tast et al., 2000) and GnRH-antagonist treatments (Virolainen et al., 2003) that caused a decline in LH lasting longer than 48 hours eventually interrupted CL function and terminated pregnancy in some of the animals. The disruption of pregnancy occurred after a time lapse of up to 2–4 weeks (Peltoniemi et al., 1995). This time lapse might be related to the intraluteal circuit of progesterone production. After starting the circuit (LH docking onto the receptor), the circuit is self-sustaining and releases progesterone. Only after a long enough absence of LH stimulation is the circuit interrupted and progesterone no longer produced.

3.3.4 Counter-current diffusion system

The ovary and the uterus can be regarded as a single entity in terms of the endocrinological processes of the cycle and pregnancy. The processes in these two organs require upstream and downstream communication. Ovarian progesterone is produced and leaves the ovary in the venous blood flowing out into the branches of the ovarian vein and in lymph via the para-ovarian lymphatic plexus and to the nearest local lymphatic node. Molecules may be transferred directly from the ovarian venous blood into the ovarian arterial blood (counter-current or local retrograde transfer) due to the difference in concentration as well as diffusion indirectly from the ovarian lymph. The ovarian artery lies in close association with the utero-ovarian vein (Figure 5) and local thinning of the wall of the vein and artery at the level of their direct contact has been examined histologically (Krzymowski et al., 1986). The structure of steroid hormone molecules facilitates their penetration of vessel walls. Small, lipophilic steroid molecules relatively easily overcome the barriers posed by the walls of lymphatic and blood vessels (Krzymowski and Stefanczyk-Krzymowska, 2012). By avoiding the systemic circulation, dilution and denaturing of molecules is avoided. About one tenth of progesterone diffuses into the ovarian lymph, mesovarial capillaries and small venous vessels based on the concentration gradient into the utero-ovarian arterial blood (Wasowska and Stefanczyk-Krzymowska, 2009). This local retrograde system allows the local destination of progesterone, which was demonstrated in higher progesterone concentrations in the blood of the uterine artery than of the vena jugularis (Stefanczyk-Krzymowska et al., 1998). The same principle applies to PGF2alpha from the uterus (Figure 5). About 40% of the uterine PGF2alpha outflowing with the uterine venous blood and lymph is transferred back in a retrograde manner into the arterial blood (Krzymowski et al., 1986).



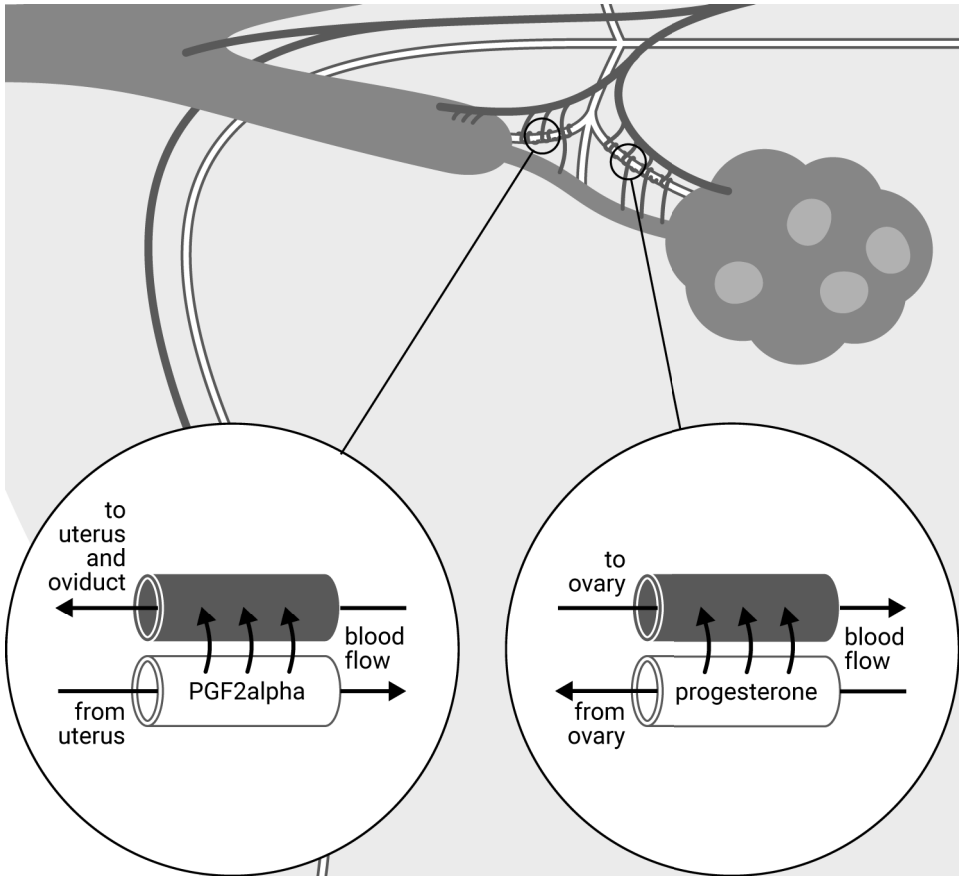


Figure 5 Counter-current diffusion system. PGF2a produced in the uterus is retrograde transferred in the mesometrial vasculature from the venous and lymphatic outflow to the arterial blood supplying the uterus and oviduct. The same principle applies to progesterone. The branches of the ovarian vein lie adjacent to branches of the ovarian artery and the blood flows in opposite directions. The concentration of steroids in the venous blood draining the ovary is, on average, 10- to 20-fold greater than that in the systemic arterial blood entering the ovarian artery and progesterone may permeate from the branches of the ovarian vein into the branches of the ovarian artery.

3.4 Uterus of the cycle and of the early pregnancy

3.4.1 Uterus of the cycle – Luteolysis

Luteolysis towards the end of the luteal phase is the process needed to enable a new ovulation and increase the chance for reproduction. The absence of embryo signals in the oestrus cycle seems to be the trigger that starts luteolysis (Senger, 2012). Bilateral hysterectomy in the mid-luteal phase of the oestrus cycle resulted in the maintenance of the CL until the end of the normal pregnancy period (Anderson et al., 1961). The luteolytic factor transporting the message "no embryos – disruption of CL function" seems to be PGF2alpha (Ziecik, 2002) that is produced in the endometrium. The secretion of PGF2alpha in the endometrium is similar in pregnant and non-pregnant gilts at Day 13– 15 after ovulation, but in pregnant gilts, PGF2alpha concentration in the utero-ovarian vein is significantly lower (Frank et al., 1977; Shille et al., 1979). Secretion of PGF2alpha is pulsatile and during the late luteal phase, as the end of the luteal phase approaches the frequency and amplitude of these pulses increase (Moeljino et al., 1977; Christenson et al., 1994).
Oxytocin, tumour necrosis factor alpha (TNFalpha) and LH might be potential modulators of endometrial prostaglandin production (Carnahan et al., 1996; Wuttke et al., 1998; Blitek and Ziecik, 2005). In the aging CL near luteolysis leukocytes (Hehnke et al., 1994; Zhao et al., 1998) are found in a growing number and the luteal sensitivity to PGF2alpha is associated with the infiltration of macrophages (Chang et al., 2017) (Figure 6). TNF2alpha derived from the macrophages inhibits the oestrogenic luteotropic machinery, interacts with PGF2alpha and leads to a decrease of progesterone production (Okano et al., 2006).
Ziecik et al. (2018) developed a "two-signals-switch hypothesis" to highlight the role of PGF2alpha during regression or rescue of the CL. Up to Day 12, PGF2alpha amplifies LH-stimulated cAMP accumulation. The "luteolytic switch" is turned on after acquisition of luteolytic sensitivity and leads to an inhibition of cAMP accumulation and to luteolysis. Oestradiol and PGE2 of embryonic and endometrial origin turn on the "rescue switch" resulting in the maintenance of steroidogenesis and cell survival.

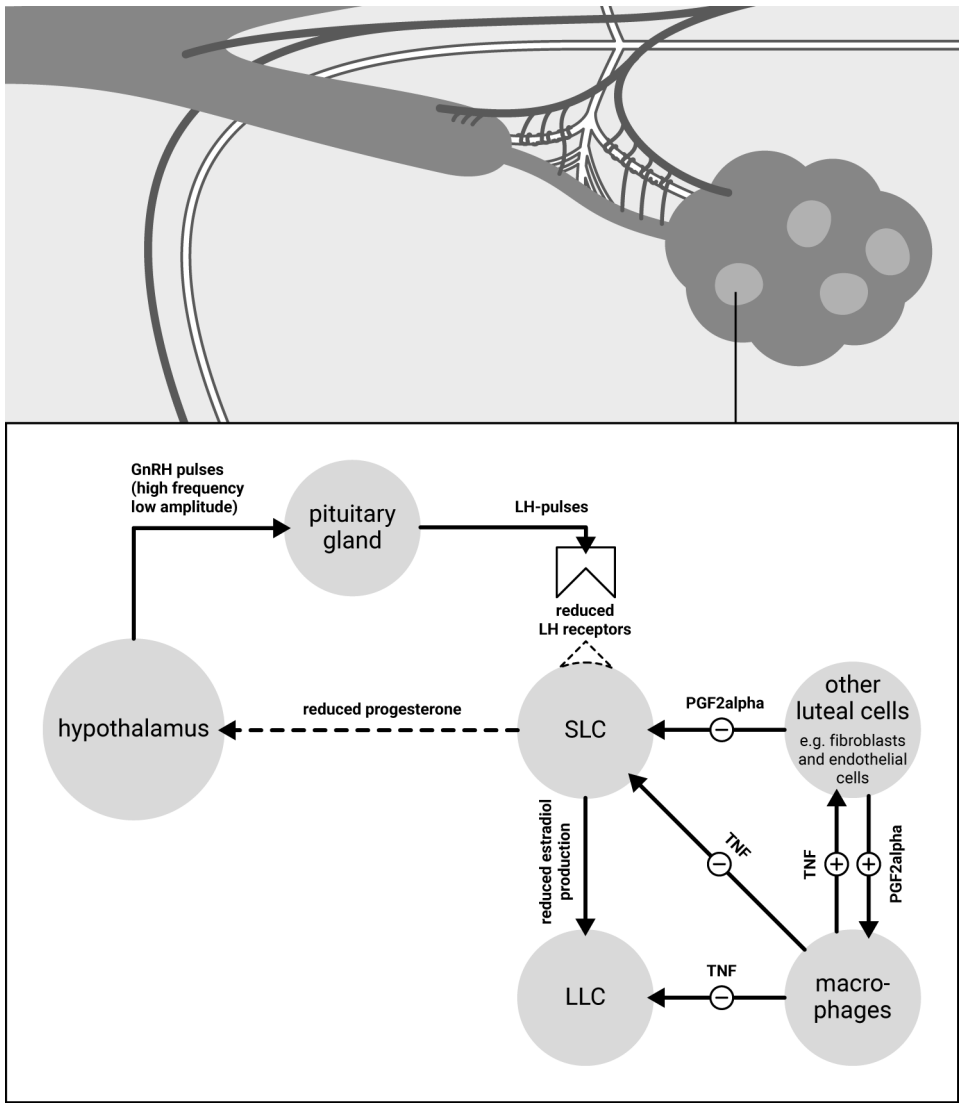


Figure 6 Tumour necrosis factor (TNF) released by invading macrophages during the late luteal phase reduces oestrogen sensitivity and inhibits the luteotropic oestrogenic machinery. TNF stimulates luteal fibroblast prostaglandin F2alpha (PGF2alpha) release, which in turn stimulates macrophage TNF release. Both TNF and PGF2alpha have direct inhibitory effects on progesterone secretion. Another action of TNF is the reduction of luteinizing hormone (LH) receptor synthesis; eventually LH pulses no longer stimulate oestrogen and progesterone release. This reduces beta-endorphin release in the hypothalamus, which in turn accelerates the GnRH pulse generator. (LLC= large luteal cells, SLC= small luteal cells).

3.4.2 Uterus of the pregnancy – maintenance of CL

PGE2 and oestrogen have been established as primary conceptus signals that prevent luteolysis in the pig (Waclawik et al., 2017). PGE2 secretion increased in utero-ovarian blood draining from the gravid horn of unilateral pregnant pigs on Day 11 to Day 12 (Christenson et al., 1994). Due to the enhanced progesterone concentration of the ipsilateral CL, PGE2 was thought to be luteotropic (Christenson et al., 1994) and it had a luteotropic effect on large luteal cells (about 9 days from oestrus) in vitro (Richards et al., 1994). Moreover, continuous intra-uterine infusions of PGE2 beginning on Day 7 after ovulation delayed luteolysis until the infusions were stopped on Day 23 (Akinolosotu et al., 1988). Both the conceptus and endometrium synthesize increased amounts of PGE2 before implantation (Waclawik et al., 2006). The expression of microsomal PGE2 synthase-1 was observed in spherical conceptuses from Day 10 onwards and it increased in tubular conceptuses at Day 13 (Waclawik and Ziecik, 2008). The porcine myometrium secretes more PGE2 than PGF2alpha during early pregnancy (Franczak et al., 2006). On Days 10–13 of gestation, the PGE2/PGF2alpha ratio is increased in the uterine lumen and vein (Davis and Blair, 1993) and in trophoblastic tissue (Waclawik and Ziecik, 2008). On Day 14 of pregnancy there is a much higher luteal concentration of PGE2than on the same day of the oestrous cycle in not inseminated gilts (Przygrodzka et al., 2015) and a higher PGE2 content only in CLs ipsilateral to the gravid horn of unilaterally pregnant gilts (Wasielak et al., 2008).
Embryonic oestradiol is supposed to trigger the rerouting of PGF2alpha. Oestrogen causes increased endometrial prolactin receptor numbers and prolactin changes the ionic flux for calcium (Geisert et al., 1982). These prolactin receptors have been detected six hours after oestradiol treatment in pseudopregnant gilts as well as in early pregnant gilts (Young et al., 1989). Prolactin increased PGF2alpha secretion from the luminal surface of the endometrium but decreased PGF2alpha secretion from the myometrial surface after oestradiol valerate treatment (Gross et al., 1990). The cause for the rerouting to the uterine lumen might be that prolactin changes the flux for calcium. Young et al. (1989) demonstrated that gilts exhibiting decreased prolactin in their blood have decreased calcium release into the uterine lumen. The calcium secretion of the endometrium into the uterine lumen as a response to oestrogen appeared only between Day 10 and Day 14 after oestrus. Gross et al. (1990) infused calciumionophore into the endometrium in vitro. The calciumionophore, which is a vehicle for the transport of

calcium across the endometrium, induced a change in the orientation of PGF2alpha from the myometrium side to the luminal side. This change was terminated when the infusion stopped and PGF2alpha returned to pretreatment endocrine-orientated secretion levels (Gross et al., 1990). Once PGF2alpha is secreted into the uterine lumen (exocrine) it cannot contact the CL and thereby prohibits luteolysis (Bazer et al., 1977). An additional anti-luteolytic mechanism may involve retrograde transfer of PGF2alpha from uterine venous blood and lymph into the uterine lumen, resulting in PGF2alpha accumulation in the veins and arterial walls of the uterus (Krzymowski and Stefanczyk-Krzymowska, 2012).

3.4.2.1 Uterus of the pregnancy – maintenance of CL: oestrogen

The idea of conceptus-derived signalling of its own presence has been discussed for decades (Geisert et al., 1982; Pusateri et al., 1996; Ziecik, 2012) and resulted in the following model. After reaching the uterine horn, conceptuses develop from a four-cell form into a thread-like form and elongate to a filamentous form at approximately Day 12 of pregnancy (Geisert et al., 1982). At Day 12, several developmental stages can be observed. The more developed embryos are thought to release oestrogen first and a critical stage of development (> 8mm or filamentous) has to be reached in order to make the embryo capable of producing oestrogen (van der Meulen et al., 1988). During morphological changes from the spherical to the tubular form at Days 11 and 12, oestrogen production per embryonic cell seems to be most intense (Pusateri et al., 1990). Oestrogens have been localized in the trophectoderm and endoderm and the highest intensity of production seems to be in the yolk sac endoderm on Days 12, 14 and 16 (King and Ackerley, 1985). Free oestrogens of embryonic source are conjugated into a biologically inactive form, mostly oestrone sulphate, and then transferred into the maternal circulation. The concentrations of oestrone sulphate increase between Day 16 and Day 30, decrease to basal level by Day 45, increase again slightly until Day 60, and then increase steadily to parturition (Robertson and King, 1974; Knight et al., 1977). The endoderm itself has some low capacity to produce oestrogens during early pregnancy (Fischer et al., 1985). At least two embryos have to be present in each uterine horn between Days 10 and 12, and sufficient embryogenic oestradiol must be produced (Dhindsda and Dziuk, 1968) to prevent luteolysis. Also, embryonic oestradiol will change uterine secretions and thereby the

uterine environment in such a way that the least developed embryos cannot develop anymore and are eliminated (Pope, 1988). Geisert et al. (1987) administered exogenous oestradiol benzoate at Day 11 and from Day 14 to Day 16 to induce pseudopregnancy, which lasted longer than 60 days in cyclic gilts. Injections of oestrogen at Day 12 and Day 13 of the oestrus cycle resulted in luteal maintenance and an extension of the interoestrus interval (short pseudopregnancy) of about 27 days, whereas an interoestrus interval of over 50 days (long pseudopregnancy) was induced if exogenous oestrogen was administered continuously from Day 12 to Day 19 (Pusateri et al., 1996a). Pusateri et al. (1996 a and b) therefore concluded that there is a biphasic maternal recognition based on embryonic oestrogen production. The first phase is the rescue of the CL and the second its maintenance.

A recent study by Meyer et al. (2019) suggested that embryonic oestrogen production might not be essential for the establishment of early pregnancy. For the study, embryos were modified in such that they lost their ability to produce oestrogen and were then transferred into the uterus of gilts. The gilts maintained pregnancy until Day 24, but beyond Day 24 gilts aborted if they were pregnant with the modified embryos (Meyer et al., 2019). That study probably just represents the beginning of discussing the maternal recognition of pregnancy in a new light.

3.5 Progesterone

Progesterone is produced in large amounts in the ovaries by the CL and in smaller amounts by the adrenal glands (Holzbauer and Newport, 1969). To a lesser extent, progesterone is produced in nervous tissue, especially in the brain (Wu and Burnham, 2018), and in adipose tissue (Kershaw and Flier, 2004). In women, the placenta maintains progesterone production during pregnancy. Progesterone synthesis in the porcine oviduct (Martiniyak et al., 2018) and uterus (Wojciechowicz et al., 2013) in vitro was also demonstrated.
Synthesis of progesterone receptors is dependent on the previous tissue exposure to oestrogens. In the uterus, progesterone will inhibit mitosis of the endometrium, induce stromal differentiation, stimulate glandular secretion in association with the accumulation of basal vacuoles in the glandular epithelium, change the pattern of proteins secreted by endometrial cells and induce quiescence of the myometrium (Niswender et al., 2000).
Measurement of ovarian progesterone release is performed by single venipuncture of the ovarian pedicle (Pharazyn et al., 1991) or by inserting a catheter into the uterine artery branch (Stefanzyk- Krzymowska, 1998) or into the utero-ovarian vein (Magness et al., 1985). Microdialysis, developed by Jarry et al. (1990), enables the study of the secretory dynamics of CL in freely moving gilts. The microdialysis system is implanted into corpora lutea and functions like an artificial capillary that has exteriorized inlets and outlets, allowing continuous perfusion. Substances that are secreted by the CL diffuse into the microdialysis fluid and are measured in the effluent fractions (Jarry et al., 1990).
All of these measuring systems require surgery and opening of the abdominal cavity. A minimally invasive method was developed by Benoit and Dailey (1991). A catheter was inserted into the vena saphena lateralis and pushed forward under anaesthesia. Ovarian and uterine veins drain into the utero-ovarian vein. Both utero-ovarian veins from right and left drain into the caudal vena cava. The tip of the catheter ends in the vena cava caudalis proximal of the affluent blood from the utero- ovarian vein. Therefore, it is possible to measure local secretion from the ovaries and from the uterus in the vena cava caudalis.

3.5.1 Progesterone secretion during the luteal phase and early pregnancy

In both cyclic and pregnant gilts, peripheral progesterone concentration increases up to Day 14 after oestrus (King and Rajamahendran, 1988). In cyclic gilts, progesterone subsequently decreases and reaches basal levels by Day 19 (Soede et al., 2011). In pregnant gilts, progesterone concentration also declines after Day 14 and reaches a relatively constant lower level around Day 20 (King and Rajamahendran, 1988; Tast et al., 2002). In the vena cava caudalis, basal progesterone in pregnant gilts rose from Day 11 to Day 13 and declined until Day 17 to below the Day 11 concentration (Brussow et al., 2011). The decline coincides with the second embryonic oestrogenic signal (Geisert et al., 1982) but it might be not related to conceptus signalling because pseudopregnant gilts seem to experience a similar decline around this time (Ziecik et al., 1986). It was speculated that there might be a signal necessary from the CL to initiate PGF2alpha release from the endometrium and the infrequent PGF2alpha pulses might prevent the CL from achieving their maximum secretory potential and reduce slightly their production in the third week (King and Rajamahendran, 1988).
It is thought that progesterone is needed and consumed by the uterus and embryos during pregnancy. Already at Day 4 of pregnancy peripheral plasma progesterone was lower in ovariectomized gilts treated with various doses of progesterone than in the not pregnant control group (Bazer et al., 1982). Hysterectomized gilts had higher progesterone concentrations in the femoral artery than early pregnant gilts (Magness et al., 1986) and they had the highest progesterone values from Day 9 to Day 27 compared to gilts with a uterus (King and Rajamahendran, 1988). In blood that has not yet passed the liver, the progesterone content was higher in veins draining the ovary than in veins draining the uterus (Pharazyn et al., 1991). Countercurrent transfer enables progesterone to enter the uterine artery where it was higher than in the uterine vein (Knight et al., 1977). Both the endometrial tissue and the conceptus metabolize progesterone, as does the endometrium of pseudopregnant gilts at Day 25 in vitro (Fischer et al., 1985).
In early pregnancy, synchronous development of the uterine environment and embryo development is essential for a high embryo survival rate (Dziuk, 1985). As changes in the oviduct (Buhi et al., 1990) and uterine environment (Roberts et al., 1987; Roberts and Bazer 1988) are largely dictated by progesterone, the timing and pattern of the rise in progesterone concentrations may be an important factor to determine embryo survival chances. Histotrophs, such as uteroferrin, formerly

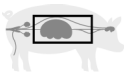
named purple protein because of its colour, and retinol-binding protein are thought to feed the embryos (Roberts et al., 1986). In ovariectomized gilts, long-term progesterone treatment markedly increased the amount of retinol-binding protein in uterine secretions (Clawitter et al., 1989).

3.5.2 Progesterone and nutrition

Numerous attempts and studies have been conducted to improve embryo survival by influencing progesterone concentration. A review by Leal et al. (2019) included 16 studies to compare effects of post-insemination energy content of feed on embryonic survival in pigs. Pigs were grouped either into gilts or sows. Altogether, 75% of the studies did not report any negative effect of feeding diets with greater energy content than that needed for body maintenance during early gestation on embryo survival. In six of the 13 studies that were conducted with gilts, high feeding level had an effect on progesterone concentration. Interestingly, the older studies reported negative effects of diets with energy contents greater than that needed for body maintenance. The authors concluded that this might be partly a result of continuous genetic selection and current improved management. Since the oldest study of this review dates back to 1981, it is questionable if the correct date of ovulation was accessed and if that may add to the confusion. Ashworth et al. (1989) and Pharazyn (1992) demonstrated that progesterone concentration and early embryonic survival might be associated in the ewe and in the gilt. Whether high plane nutrition negatively effects porcine embryonic survival is unclear (Cassar et al., 1994) and might depend on the timing of changes in feed intake (Pharazyn et al., 1991). It might be that not only the feeding level but also the dietary energy source affects peripheral progesterone concentration and that the metabolic clearance rate might be the reason for altered progesterone concentration (Athorn et al., 2011) (Figure 7). Reduced feed intake may be associated with reduced blood flow to the liver via hepatic portal circulation (Prime and Symonds, 1994), reduced sequestration of steroids in the gut contents and entero-hepatic recirculation (Miller et al., 1999). Quesnel et al. (2010) showed that embryo survival is not affected by a high level of feeding in the modern highly prolific gilt with ovulation rates of 20. It might be that due to the high ovulation rates progesterone concentration has also increased compared with 40 years ago. A high level of feeding might by itself still have negative impacts on embryo

survival, but the higher intrinsic progesterone concentration could mask that.

The research of Virolainen et al. (2005), in which the influence of feed intake on vena cava caudalis progesterone concentrations was studied, did not find any profound effects of the level of feeding on the vena cava caudalis progesterone concentrations at Day 22 of pregnancy in gilts. Eating, however, affected the progesterone concentration when compared within a day. The progesterone concentration in the jugular vein decreased after eating but increased slightly in the vena cava caudalis. Nonetheless, the study revealed that in order to achieve appropriate information about effects of feeding and, in general, about changes in progesterone, it might be more valuable to execute an intensive sampling from a blood vessel close to the ovary (Figure 7).



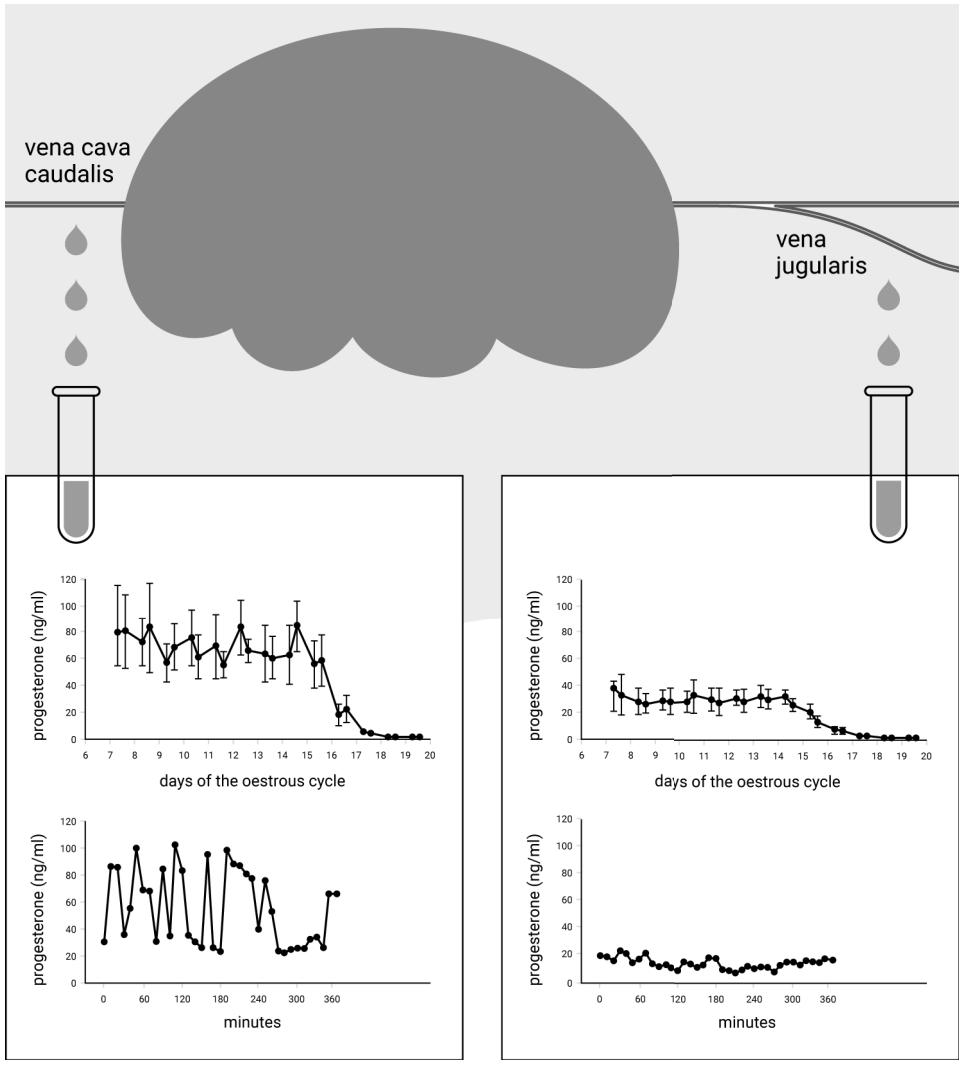


Figure 7 Metabolization of progesterone in the liver. Example of progesterone measured in the vena cava caudalis and vena jugularis during the oestrus cycle. Upper panels show the progesterone concentration of four gilts measured two times daily from Day 7 to Day 19 of the oestrus cycle and lower panels of one individual gilt measured at ten-minutes intervals for six hours on Day 12 of the oestrus cycle. Progesterone release from the ovary is episodic, as measured in blood samples drawn from the vena cava caudalis. After metabolization in the liver, mean progesterone concentration is about half of the mean concentration before metabolization and the pulse amplitude is lower or absent (modified after Brussow et al., 2008).

Langendijk et al. (2017) demonstrated the effects of fasting on Day 11 on progesterone concentration measured locally versus systemically. While progesterone concentration was not compromised by fasting on Day 11 in the vena cava caudalis, it was up to 30% lower from Day 12 to Day 15 in the vena jugularis. Fasted gilts bore fewer piglets (Langendijk et al., 2017). Another study, conducted by Athorn et al. (2013), assessed embryo survival and progesterone concentration in the vena cava caudalis on Days 6 and 9 of pregnancy in gilts that were allocated either to a high or low feeding regime from Day 0 of pregnancy onwards. The highly fed gilts tended to have a higher mean progesterone concentration on Day 6, they had more pulses on Day 9 and embryo survival was greater at Day 10. Athorn et al. (2011) studied local progesterone supply from the ovaries to the uterus and the contribution to embryo survival in gilts fed close to maintenance requirement and gilts fed more than twice the maintenance requirement. They were able to demonstrate that the embryo survival was higher in the uterine horn that was supplied locally with progesterone by an intact ovary than in the uterine horn that depended on systemic supply due to ipsilateral ovariectomy. The difference in embryo survival between the horns was more pronounced in the gilts fed at the high level and overall embryo survival at Day 35 was lower for the low feed level gilts. These studies of feeding on local progesterone concentrations and embryo survival confirm the diversity of studies addressing feeding on systemic progesterone. However, it might be a question of amount and ingredients of feed and day of pregnancy when commencing the diet that influences progesterone concentration and embryo survival.

3.5.3 Pulsatile progesterone release

Pulsatile progesterone secretion has already been demonstrated in human (Filicori et al., 1984) and bovine CL (Walters et al., 1984). Jarry et al. (1990) demonstrated that also porcine progesterone is released in pulses during the midluteal phase. Taking a series of blood samples in the conscious pig close to the ovary was done already by Parvizi et al. (1976) and Eiler (1978). Parvizi et al. (1976) found that in miniature pigs during late gestation, progesterone release in the vena cava caudalis partly followed pulsatile LH release with a 20–60 min delay. Virolainen et al. (2005) demonstrated that progesterone secretion was episodic (Figure 7) and implicated a possible association between

progesterone pulses and LH pulses in one gilt at Day 22 of pregnancy. Neither Langendijk et al. (2017) nor Brussow et al. (2011) could establish a relationship between LH release and progesterone pulsatility in the vena cava caudalis at Day 11 or between Days 11 and 17 of gestation, respectively.

The finding concerning progesterone pulses in the pig is new compared to the long history of progesterone research. It is currently completely unclear why the CL releases progesterone in a pulsatile manner because pulsatile release has been associated with neurohormones, such as oxytocin, where quick action is the modus operandi. In addition, no common definition has yet been found to track, detect and describe progesterone pulses. Even the word ‘pulse’ has not been used consistently. The different studies that have been employed to date on progesterone pulsatility in the vena cava caudalis are listed in Table 1. It is listed for what kind of purpose progesterone secretion was studied and below the table are the different definitions of the few studies that employed progesterone pulsatility.

Table 1 Studies on pulsatile progesterone (P4) release in early pregnancy.

Day of pregnancy	6	9	11	11	13	15	17	22
P4 basal (ng/ml)	37/38	45/45	-	47.6	53.1	52.8	41.3	10.3/28.2
P4 mean (ng/ml)	70/88	85/102	133	-	-	-	-	19.2/28.2
P4 pulse frequency	-	3.8/4.9	5.1	3.2	3.8	4.3	4.3	3/0
Duration, interval	6h, 15 min	6h, 15 min	6h, 15 min	6h, 10 min	6h, 10 min	6h, 10 min	6h, 10 min	12h, 30 min
LH basal (ng/ml)	-	-	0.22	0.23	0.11	0.11	0.17	0.8
LH mean (ng/ml)	-	-	0.37	-	-	-	-	1.2/1
LH pulse frequency	-	-	0.8	1	1.3	1.2	1.7	5/2
Number of embryos	11.6/14	11.6/14	10.9 born	12.8	12.8	12.8	12.8	11.5
Purpose of pig	feeding trial gilts (2 groups)	feeding trial gilts (2 groups)	control gilts	control gilts	control gilts	control gilts	control gilts	feeding trial gilts
Reference	Athorn et al. 2013	Athorn et al. 2013	Langendijk et al. 2017	Brussow et al. 2011	Brussow et al. 2011	Brussow et al. 2011	Brussow et al. 2011	Virolainen et al. 2005
Definition basal P4	Mean of three consecutive samples preceeding every suspect pulse							
Definition P4 pulse	At least two consecutive samples, one of which was greater than 100g/ml and the other that was greater than basal concentration			At least two consecutive samples that exceeded the basal level by more than three standard deviations and in which the peak concentration was completed within two subsequent intervals				

4

Aims of the study

This study represents an investigation into LH release pattern relative to the progesterone secretion pattern of CLs in the pig during the time window of Day 11 until Day 21 of gestation, the time of the first embryonic signal, maternal recognition and attachment in early pregnancy.
Consequently we researched LH and progesterone in gilts and primiparous sows.

Specific aims of the study are as follows:

1 To examine whether progesterone secretion pattern is responsive to LH pulses and to establish a possible relationship or association between the two hormones.
2 To investigate whether inducing an absence of LH pulses influences progesterone release pattern, and hence if there exists a dependency of progesterone pulsatile release on LH pulses.
3 To study the effect of the process of sexual maturation on the luteal support function of LH in pregnant gilts and sows.

5

Materials and methods

5.1 General information

Wording: the word ‘gilts’ is used for the nulliparous sows in Studies I and II, and ‘sows’ for the primiparous sows in Study III. When referring to all female animals (gilts and sows) in all three studies, the word ‘sow’ is used. If not stated otherwise, the results refer to pregnant sows and Day refers to the Day of pregnancy, definition for counting the Day of pregnancy is given in Table 2. The number of animals, their breed, group size, parity, housing, and the days of catheterization, sampling and euthanasia are given in the overview in Table 2.
Progesterone secretion and progesterone release are always measured in the vena cava caudalis (vcc) if not stated otherwise.
Detailed information on the materials and methods is available in the original publications (I–III); an overview is presented here of the most relevant aspects. Studies I and II were approved by the Animal Experiment Board ELLA in Finland (permission ESLH–2009–06207/Ym–23). Study III was approved by the Institutional Animal Use and Care Committee of Wageningen University (Wageningen, The Netherlands).

Table 2 Overview of animals and the management used in the three studies. Overview on materials and methods of Study I (progesterone and luteinizing hormone secretion patterns in early pregnant and non-pregnant gilts), Study II (GnRH-agonist deslorelin implant alters the progesterone release pattern during early pregnancy in gilts) and Study III (caudal vena cava progesterone and LH release patterns on Day 14 of gestation in primiparous sows).

Study number	Number of animals	Breed	Number of animals in the treatment group and control group	Parity	Housing	Day of inserting the vena cava caudalis catheter	Sampling day(s)	Day of eutha-nasia
I	14	Landrace x Finnish Yorkshire	8 (pregnant) 6 (not pregnant)	0	Individual pens 2 x 2 m, straw bedding	11	11, 16, 21 (Day 1 = day of last insemination)	22-30
II	13	Landrace x Large white	6 GnRHa (GnRHagonist treatment) 4 control (placebo treatment)	0	Individual pens 2 x 2 m, straw bedding	11	16, 21 (Day 0 = day of first insemination)	23
III	8	Yorkshire x Dutch Landrace	8 (only one group in the study)	1	Individual cages	13	14 (Day 1 = day of first insemination)	35

5.2 Animals and management

The gilts originated from two different commercial Finnish farms (Studies I, II). After they had shown oestrus at least once in their herd of origin they were transported to the research unit of the University of Helsinki in Viikki, Helsinki. The following oestrus was awaited and they were inseminated (I). The gilts of Study II were inseminated on their home farm and after insemination they were brought to the research unit of the University of Helsinki in Viikki, Helsinki. At the research unit, gilts of Studies I and II were housed in individual pens of at least 2 x 2 m with iron bars separating them from their conspecifics. The pens had straw bedding and daylight entered from windows. The pigs were fed twice a day with a commercial ration of about 3.0 kg per day (13 MJ/kg, 14.5% crude protein and 7.4 g lysine [Tiineyspekoni®, SuomenRehu, Finland]).
The primiparous sows of Study III came from a TopigsNorsvin breeding gilt farm, and were transported to the research unit of Wageningen University at Day 100 of gestation. After weaning their first litter, the sows were individually housed in gestation crates. They were fed 2.5 kg / day of a commercial lactation diet until the second day after insemination and from the third day of gestation they were fed either 2.5 kg / day (n = 5) or 3.25 kg / day (n = 3) of a commercial gestation diet (12.2 MJ ME, 13% crude protein, 0.65% lysine, Dracht Zeugenvoer, De Heus Voeders).
All animals of Studies I, II and III had free access to water.
At the end of the experiments, the gilts of Studies I and II were euthanized using intravenous medication (description of drugs in original article) and the sows in Study III by stunning and exsanguination. All sows were autopsied and the reproductive tracts of the animals were collected.
The ovaries were removed and the number of CL was counted. Uterine horns were cut open longitudinally, embryos were recovered and the viability of the embryos was evaluated by their size and appearance. Embryo survival was expressed as a percentage of viable embryos from the number of CL.

5.3 Oestrus detection and insemination

After the gilts in Studies I and II had shown at least one oestrus, their subsequent oestrus was synchronized (description of synchronization programme in original article). The sows of Study III were weaned and oestrus was awaited. Oestrus was checked twice a day while the animals had contact with a boar through the bars of the pen. If backpressure testing was positive, the gilts were inseminated after six hours. They were inseminated at intervals of 24 hours as long as they showed a standing response to the boar. Detailed information about the semen can be retrieved from the original articles.

5.4 Pregnancy

In Studies I and III, pregnancy was confirmed macroscopically at autopsy after day 20 (see Table 2). Based on the pregnancy findings at autopsy, gilts of Study I were restrospectively classified as pregnant or non-pregnant on Day 11. In Study II, pregnancy status was assessed using a grey-scale ultrasound Honda Electronics HS—1500 Vet scanning device with a 5 MHz linear probe on Days 12 and 13 (Image 1) (Kauffold et al., 2010) and confirmed with real-time ultrasound (Pie medical, equipped with a 5 MHz sector probe) on Day 21 and Day 22.

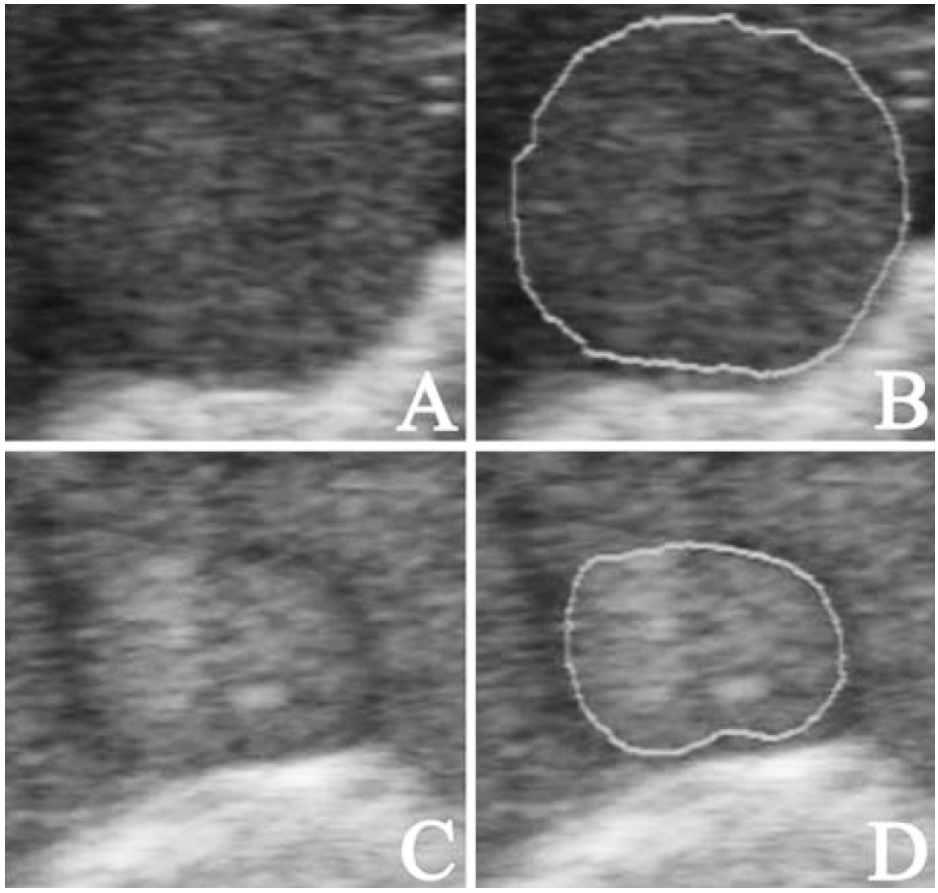


Image 1 An example of grey-scale ultrasound to detect early pregnancy in the gilt. Images A and B are from a pregnant gilt on Day 13 post ovulation (mean grey value 8.6). Images C and D are from a non-pregnant gilt on Day 11 post ovulation (mean grey value 10.2). Scale bars on the left and top margin in 0.5 cm. (Kauffold et al., 2010).

5.5 Catheters and blood collection

In all sows in all studies, a catheter was inserted into the vena saphena lateralis that ended in the vena cava caudalis. The animals were anaesthetized during the catheterization (Heinonen et al., 2009) and a PVC tube, 1.0 mm i.d., 1.5 mm o.d., Sterihealth Laboratory Products Pty LTD, Australia (Virolainen et al., 2005) was used. For the gilts, 52 cm of the tube was inserted and for the sows 61 cm was inserted. The procedure for the catheterization was taken from Virolainen et al. (2005) and can be studied in detail in the publication of Study II (Haen et al., 2019). The correct position of the end of the catheter was confirmed at autopsy. Sows of Study III were also equipped with a vena jugularis catheter. A 50 cm vein catheter was inserted into the ear vein and pushed forward on Day 13 after insemination (Peacock, 1991).
The catheters were flushed daily to avoid clotting of blood in the tubes or at their tips in the blood vessel. With that aim, a syringe was attached to the free end of the tube, 5 ml of blood were drawn and discarded and the catheter was flushed with heparinized NaCl solution. On the days of intensive blood sampling, the first 2 ml of blood were discarded and a 5 ml (Studies I and II) or a 3 ml (Study III) blood sample was drawn. After each sampling, the catheter was flushed with heparinized NaCl solution. The collected blood sample was transferred into lithium heparin tubes (Studies I and II) or in EDTA tubes and placed on ice (Study III) and centrifuged at 1800g for 10 min (Studies I and II) or 3000g for 10 min (Study III) within one hour. Plasma was stored at -20°C until the day of laboratory analysis.

5.6 Gonadotropin hormone agonist

For Study II, an implant was inserted on Day 11 while the gilts were under anaesthesia for the vena cava caudalis catheterization. The implant was either impregnated with 4.7 mg deslorelin (Suprelorin®, Inc. Virbac, France) (n = 8) for the GnRH agonist group (GnRHa group) or not impregnated (n = 5) (placebo) for the control group. The implant was placed intramuscularly in the neck, about 5 cm caudal from the base of the ear (Kauffold et al., 2010). The implant released deslorelin slowly at a rate of approximately 20 µg/day (Navarro, 2011). The release initially led to a stimulation of LH and FSH for some hours and had thereafter a down-regulating effect on LH secretion (Trigg et al., 2001).

5.7 Hormone assays

Detailed procedures for the hormone assays are published in the original articles, an overview is given here.

5.7.1 Progesterone

For Studies I and II, the blood samples were analysed for progesterone using a direct commercial radioimmunoassay (RIA) (Spectria®, Orion Diagnostica, Turku, Finland) that had been validated to measure progesterone in pig plasma (Peltoniemi et al., 1995). For Study III, the plasma progesterone concentrations were analysed in duplicate using a commercial Coat- A-Count P4 RIA kit (PITKPG-7®; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA).

5.7.2 Luteinizing hormone

For Studies I and III the LH concentrations were analysed in duplicate, using homologous double-antibody radioimmunoassay according to the method described by Cosgrove et al. (1991), with some modifications as described by Hoving et al. (2012a). Study II LH analyses were performed using an enzyme-linked immunosorbent assay (LH Detect® , ReproPharm, INRA, Paris, France) developed and previously used for porcine plasma (Norrby et al., 2011).

5.7.3 Hormone parameters definition

For LH and progesterone, mean level was defined as the average concentration of all samples (I, II, III). Basal level was defined as the mean of six (III) or eight (I, II) samples with the lowest hormone concentration.
A progesterone pulse started when at least two consecutive samples exceeded the basal concentration by more than two (III) or three (I, II) standard deviations and subsequent values above this threshold belonged to the same pulse. An LH pulse started when LH levels

exceeded the basal concentration by more than two standard deviations (I, II, III) (Tast et al., 2000; Langendijk et al., 2017). Subsequent values above this threshold were deemed to belong to the same pulse. Pulse amplitude was reached within two samples from the previous nadir and there were at least two samples between the pulse amplitude and the return to basal level or the next nadir. For both LH and progesterone, the pulse amplitude was the difference between the maximum pulse value minus basal level. Mean pulse amplitude was the average of the pulse amplitudes for each sow on each sampling day. Further, a progesterone pulse following an LH pulse within one hour was regarded as a response to the LH pulse (I, III).

5.8 Statistical analyses

The statistical tests for Studies I and II were conducted using IBM® SPSS® statistics versions 24 and 25 and for Study III, the statistics program of SAS Institute, Cary, NC, USA was used. Data were analysed by repeated measurement analysis of variance (split-plot ANOVA, Gill and Hafs, 1971) using the general linear model with repeated measures. Outcome variables were basal concentration, mean concentration and number and amplitudes of pulses of progesterone and LH on Days 11 (I), 16 (I, II) and 21 (I, II). The within-subject factors were Day 11, 16 and 21 for progesterone or Days 11 and 21 for LH in Study I. In Study II, treatment was the between-subject fixed effect, and period (day, clock time) was considered as the within-subject repeated factor. Differences in LH parameters between Day 11 and Day 21 and between progesterone parameters at Day 11, Day 16 and Day 21 in Study I and between vena jugularis and vena cava caudalis progesterone concentrations in Study III were evaluated with pairwise t-tests. Possible relationships between LH and progesterone outcome variables and number of CL and embryos were examined using Pearson's correlation coefficients (I, III).

6

Results

6.1 Macroscopic examination of the reproductive organs

Autopsy of the reproductive organs revealed a pregnancy rate of 57% (8/14) (Study I), 80% (4/5) and 83% (5/6) (control and GnRHa group, respectively, Study II) and 100% (8/8) in Study III. The numbers of CL of the pregnant gilts of Studies I and II were 16.25 ± 5.0 and 17.3 ± 4.3, respectively and were 22.5 ± 3.7 in the sows of Study III (mean ± SD). On none of GnRHa gilts' ovaries (n = 5) of Study II were follicles detected and two of the five gilts had a cyst of 15 mm on one ovary, besides the present CL (Image 2). The numbers of embryos were 14.0 ± 4.75 (I), 10.4 ± 4.8 (II) and 14.8 ± 2.9 (Study III) and embryo survival rate was 0.89 ± 0.24 (Study I), 0.6 ± 0.32 (II) and 0.69 ± 0.18 (Study III).

Image 2 The ovary of a gilt on Day 23 of pregnancy that was treated with a GnRH slow- release agonist on Day 11 and developed a cyst next to the CL.

6.2 Luteinizing hormone

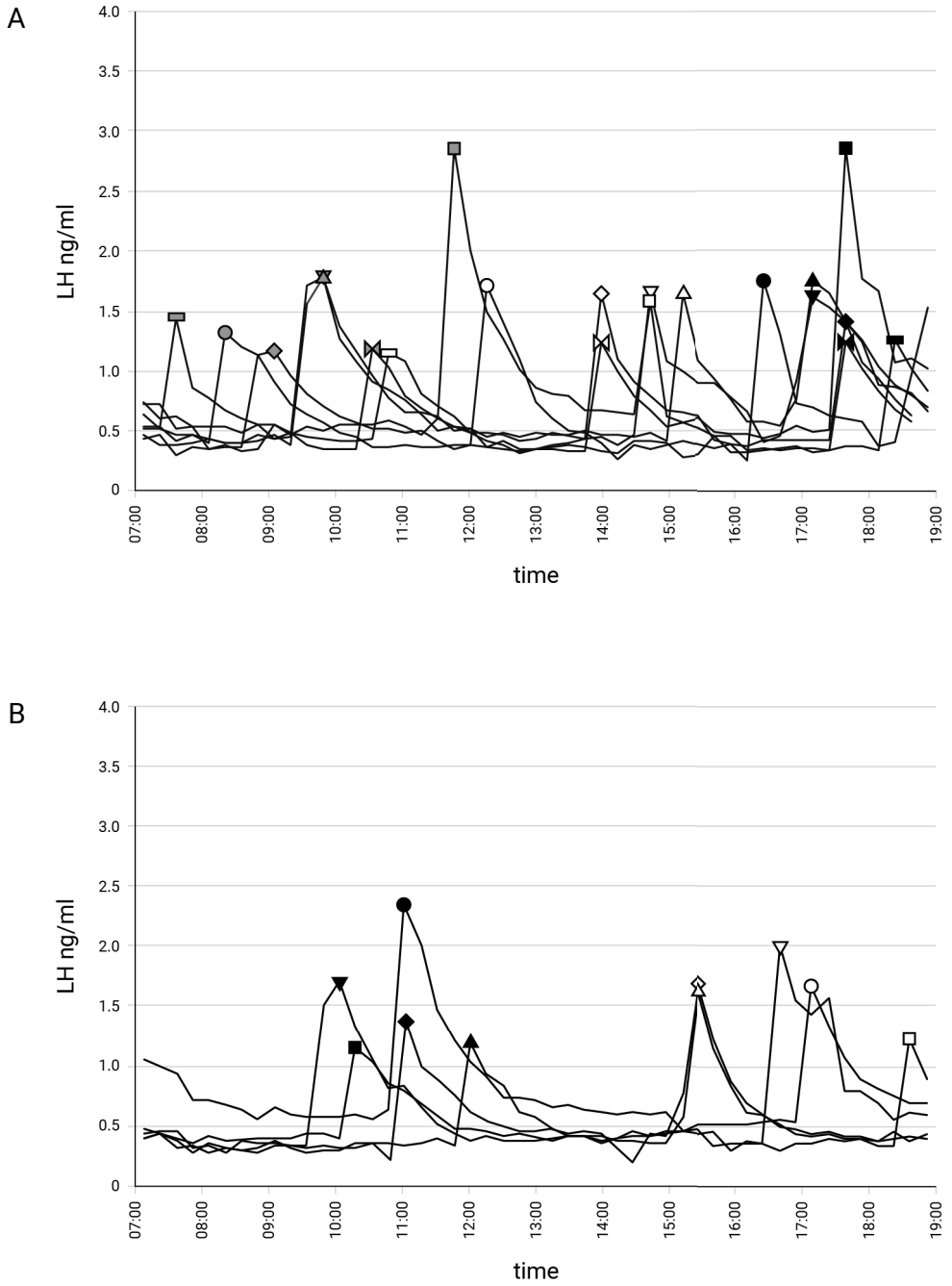
In Studies I and II we observed differences in LH parameters between the groups. In Study II, LH pulsatility was effectively terminated in the GnRHa group on Days 16 and 21 of pregnancy (treatment effect: $p < 0.01$) (Table 3). In Study I, non-pregnant gilts tended to have a lower LH pulse frequency (1.17 ± 1.33 vs. 2.30 ± 0.51; $p = 0.07$) and mean LH pulse amplitude (0.72 ± 0.92 vs. 1.78 ± 0.57; $p = 0.08$) than pregnant gilts on Day 11 (Table 3). A significant decrease in LH pulse amplitude was observed from Day 11 to Day 21 (from 1.78 ± 0.57 ng/ml to 1.50 ± 0.32 ng/ml) in the pregnant gilts.

LH parameters of the pregnant untreated sows in Studies I, II and III.				
Study, Day	**Basal ng/ml**	**Mean ng/ml**	**Pulse amplitude**	**Pulse frequency**
I, 11, n=6	0.35 ± 0.07	0.64 ± 0.14	1.78 ± 0.57^{ax}	2.30 ± 0.51/12h^{x}
I, 21, n=6	0.37 ± 0.08	0.63 ± 0.14	1.50 ± 0.32^{b}	2.67 ± 0.52/12h
II, 16, n=5	0.55 ± 0.59	0.62 ± 0.60	1.24 ± 0.53^{c}	1.20 ± 0.45/8h^{c}
II, 21, n=4	0.57 ± 0.98	0.73 ± 1.01	1.03 ± 0.73^{c}	1.40 ± 0.45/8h^{c}
III, 14, n=8	0.40 ± 0.10	0.70 ± 0.10	1.00 ± 0.20	4.30 ± 1.50/10h

LH parameters of the non-pregnant gilts of Study I and of the GnRH agonist treated gilts of Study II.				
Study, Day	**Basal ng/ml**	**Mean ng/ml**	**Pulse amplitude**	**Pulse frequency**
I, 11, n=6	0.42 ± 0.17	0.61 ± 0.23	0.72 ± 0.92^{y}	1.17 ± 1.33/12h^{y}
II, 16, n=6	0.27 ± 0.21	0.50 ± 0.25	0^{d}	0/8h^{d}
II, 21, n=5	0.20 ± 0.14	0.50 ± 0.22	0^{d}	0/8h^{d}

Table 3 LH parameters for Studies I, II and III. Data presented as means ± SD. Different superscript letters denote statistically significant differences. ^{ab} denotes differences between days in pregnant animals, $p \leq 0.05$. ^{cd} denotes differences between the GnRH a group and the control group within the column, $p \leq 0.05$. ^{xy} denotes differences between pregnant and non-pregnant animals within the column, $p \leq 0.10$.

In Study I, we combined pregnant gilts' LH results for Day 11 and Day 21. That way we achieved 12 LH release patterns independent of the day. We observed that the gilts had three (n = 7) or two (n = 5) LH pulses in 12 hours. When the gilts had three pulses, the pulses fell into three distinct periods (between 07.30 h to 10.30 h, 12.15 h to 15.15 h and 16.30 h to 18.30 h) (Figure 8a). On average, one pulse period lasted 113 ± 38 minutes. When gilts had two LH pulses, they fell into periods between 10.00 h to 12.00 h and 15.30 h to 18.45 h (Figure 8b and Figure 9). On average, their pulse period lasted 79 ± 62 minutes.



Figures 8 Study I. LH patterns in a 12-hour (07:00-19:00) sampling period combined for Day 11 and Day 21 of pregnancy in gilts, same amplitude marker is for the same gilt.

A LH secretion pattern of gilts that have a frequency of three pulses in 12 hours; first pulse period: grey markers, second pulse period: white markers, third pulse period: black markers.

B LH secretion pattern of gilts that have a frequency of two pulses in 12 hours; first pulse period: black markers, second pulse period: white markers.

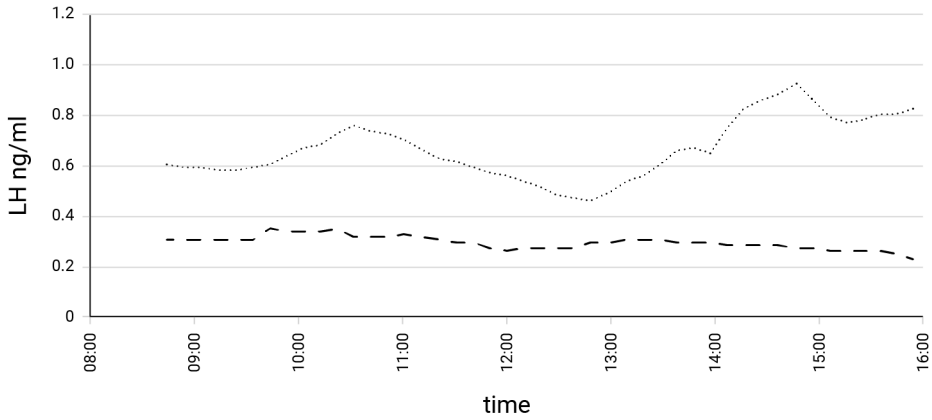


Figure 9 Study II. Luteinizing hormone (LH) moving average values (average of five values) of deslorelin-implanted (dashed line) and control (dotted line) gilts from plasma samples collected on Day 16 of pregnancy. A sampling window of 8 hours was used with a 10-min sampling interval. The slow-release GnRH treatment significantly reduced LH secretion from 08:00 to 12:00 and 12:10 to 16:00.

6.3 Progesterone

The release pattern of progesterone measured in the vena cava caudalis was highly pulsatile in all three studies. The decline of progesterone pulse amplitude and frequency from Days 11 and 16 to Day 21 in Study I was statistically significant. Mean progesterone concentration declined significantly from Day 11 (I) and Day 16 (I, II) to Day 21 (I, II) in pregnant gilts (I: $p < 0.05$; II: $p = 0.016$) (Table 4 and Figures 10 and 11). Additionally, sampling site (III) and treatment (II) but not pregnancy status on Day 11 (I) had a significant effect on the progesterone parameters (Table 4). In Study III, progesterone parameters were compared at two sampling sites: vena jugularis and vena cava caudalis, at Day 14 of pregnancy. Mean progesterone concentration in the vena cava caudalis (65.5 ± 19.8 ng/ml) was about double the value of that in the vena jugularis (27.6 ± 1.5 ng/ml). The mean progesterone concentration of individual sows in the vena jugularis was similar (ranging from 23.8 ± 2.8 to 30.1 ± 3.4 ng/ml) whereas it differed considerably in the vena cava caudalis (ranging from 34.1 ± 9.2 to 404.0 ± 122.7 ng/ml).

In Study II mean vcc progesterone concentration differed significantly between the GnRHa group and the control group ($p < 0.001$) on Day 21. In Study I, there was no significant difference in vcc progesterone parameters on Day 11 between pregnant and non-pregnant gilts.

Table 4 Progesterone parameters for Studies I, II and III. Data presented as means ± SD. [ab] denotes differences between days in pregnant animals, $p \leq 0.05$. [y] denotes differences between the treatments within the column, $p \leq 0.05$.

Vena cava caudalis progesterone parameters of the pregnant sows in Studies I and III and the pregnant control gilts of Study II.

Study, Day	Basal ng/ml	Mean ng/ml	Pulse amplitude	Pulse frequency
I, 11, n=8	18.53 ± 1.94[a]	24.87 ± 4.70[a]	46.10 ± 12.60[a]	4.00 ± 1.85/12h[a]
I, 16, n=5	17.16 ± 5.25[a]	20.19 ± 4.86[a]	37.90 ± 6.00[ab]	3.00 ± 1.23/12h[a]
II, 21, n=6	12.57 ± 3.28[b]	13.70 ± 3.04[b]	21.90 ± 12.90[b]	2.17 ± 12.90/12h[b]
II, 16, n=5	17.88 ± 4.64	21.10 ± 5.55	30.12 ± 17.13	1.40 ± 1.14/8h
II, 21, n=4	6.98 ± 5.18	8.45 ± 4.00[y]	9.99 ± 9.38	0.60 ± 0.55/8h
III, 14, n=8	33.60 ± 13.10	65.50 ± 19.80	n.a.	4.70 ± 1.60/10h

Progesterone parameters for non-pregnant gilts (I), GnRH agonist treated pregnant gilts (II) and pregnant sows from the vena jugularis (III).

Study, Day	Basal ng/ml	Mean ng/ml	Pulse amplitude	Pulse frequency
I, 11, n=6	19.23 ± 6.08	24.92 ± 8.14	40.00 ± 8.00	3.67 ± 2.81/12 hours
II, 16, n=6	18.95 ± 7.25	24.75 ± 10.90	27.84 ± 0.00	1.00 ± 0.71/8 hours
II, 21, n=5	12.16 ± 7.29	14.42 ± 7.61[y]	14.94 ± 12.85	0.85 ± 0.75/8 hours
III, 14, n=8	n.a.	27.6±1.5	0	0

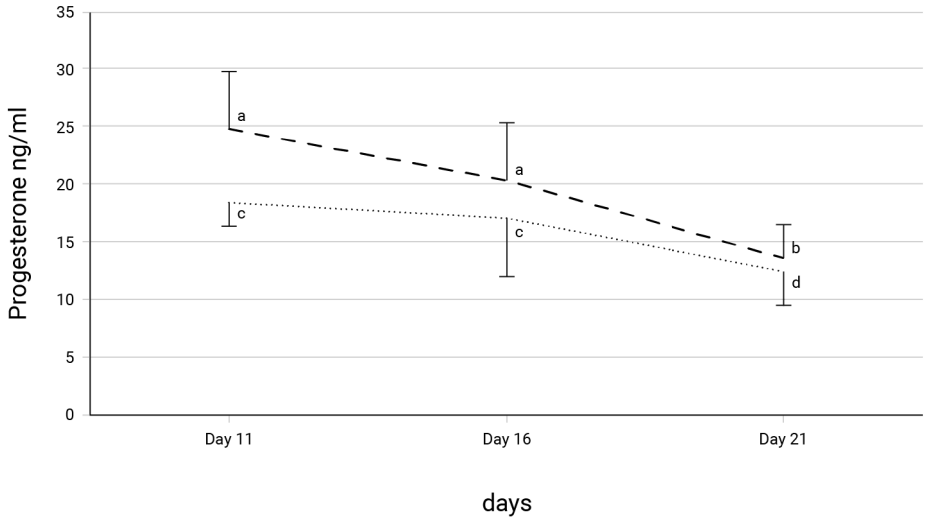


Figure 10 Mean (dashed lined) and basal (dotted line) progesterone concentration (ng/ml) on Day 11 (n = 8), Day 16 (n = 5) and Day 21 (n = 6) of pregnancy, assessed using a 12-hour sampling period with sampling intervals of 15 minutes. Data are presented as means ± SD. Means with a different data label differ significantly ($p \leq 0.05$) (Study I).

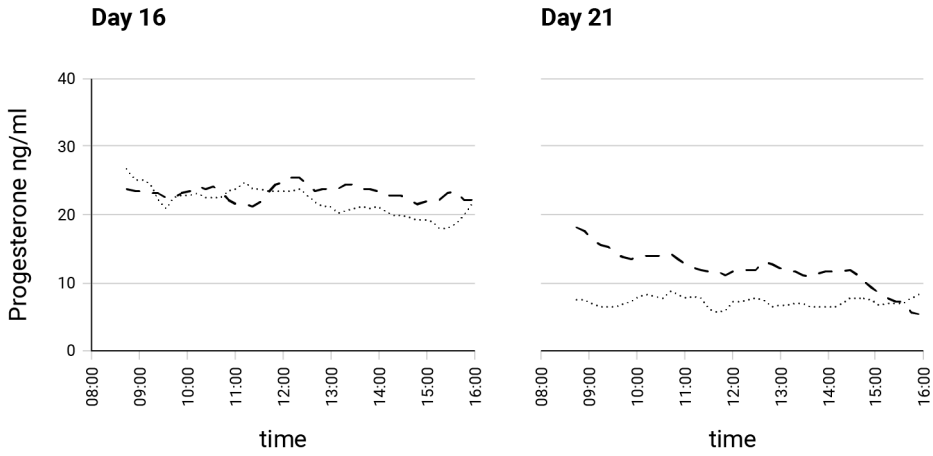


Figure 11 Gilts' progesterone moving average values (average of five values) on Day 16 and Day 21 of pregnancy, deslorelin-implanted (GnRHa group), dashed line (n = 6 and 5) versus control group, dotted line (n = 5 and 4). A sampling window of 8 hours was used with a 10- min sampling interval (Study II).

6.4 Relationship between progesterone and LH pulsatility

The percentage of LH pulses that was followed by a progesterone pulse within one hour was 21.4% (three of the 14 LH pulses) in gilts on Day 11 (I), 60.8% (14 of the 23 LH pulses) in sows on Day 14 (III) (Figure 12) and 0% in gilts on Day 16 (II) and Day 21 (I, II). Absence of LH pulsatility in gilts implanted with a GnRH agonist (II) did not impair progesterone pulsatility at Day 16 of pregnancy (Figure 13).
On Day 21, in four of the six pregnant gilts of Study I, a specific pattern in LH and progesterone secretion was observed, in which pulsatile secretion of one hormone was present, while pulsatile secretion of the other hormone was absent (Figure 14).

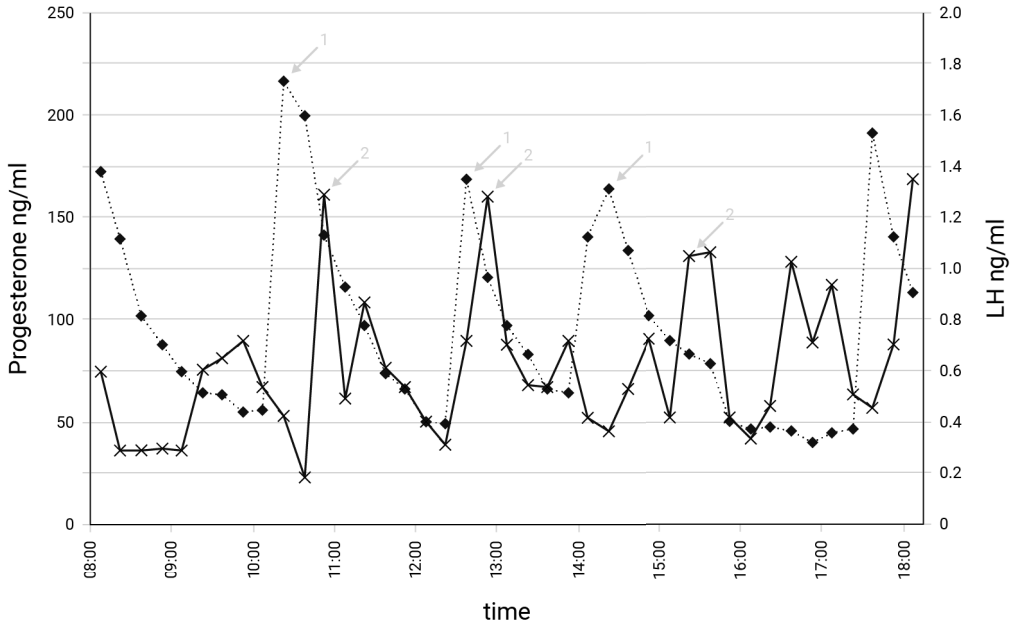


Figure 12 Study III. Hormone concentration of one pregnant sow on Day 14, showing a temporal relationship between LH and progesterone. Progesterone (solid line) and LH (dotted line) were taken at 15-min intervals from 08:00 until 18:00. An LH pulse (arrow 1) is followed by a progesterone pulse (arrow 2) within an hour.

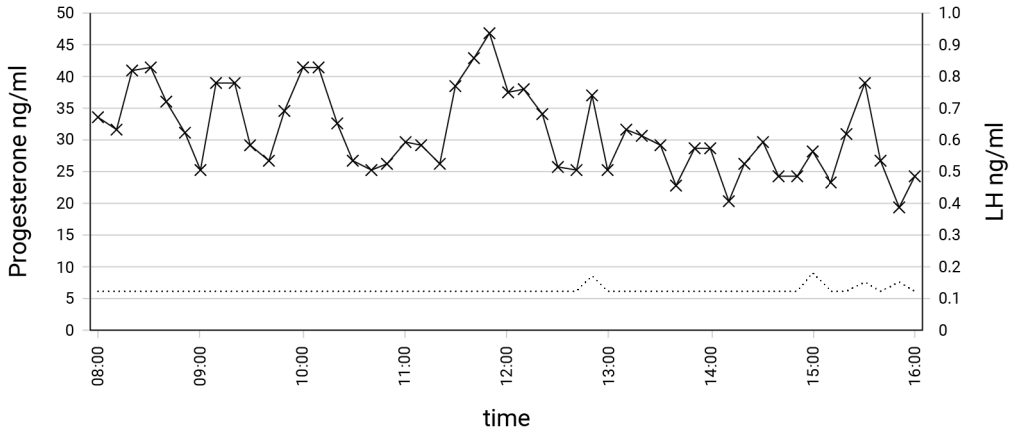


Figure 13 Study II. Progesterone (solid line) and LH (dotted line) taken at 10-min intervals from 08:00 until 16:00 on Day 16 of pregnancy in one gilt treated with a slow-release GnRHagonist on Day 11. Although there are no LH pulses, progesterone secretion pattern is pulsatile.

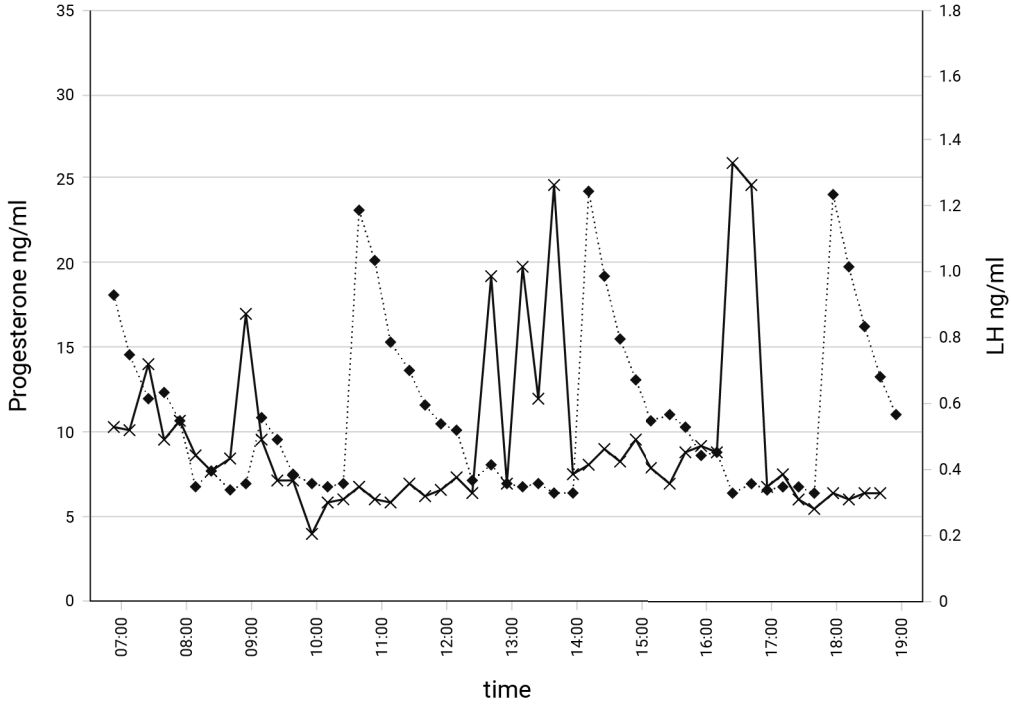


Figure 14 Study I. Progesterone (solid line) and LH (dotted line) taken at 15-min intervals from 07:00 until 19:00 on Day 21 of pregnancy in one gilt. Progesterone pulsatility is seen when LH secretion is basal.

6.5 Correlations of LH and progesterone with embryo and CL numbers

The number of CL was 16.25 ± 5.0 (Study I) and 17.05 ± 3.8 (Study II) in gilts and 22.5 ± 3.7 in sows (Study III). The number of CL was positively correlated with the number of progesterone pulses on Day 14 of pregnancy in sows ($r = 0.87$; $p = 0.023$) (III). In pregnant gilts, the CL number was negatively correlated with mean progesterone concentration ($r = - 0.78$, $p = 0.02$), number of progesterone pulses ($r = -0.80$, $p = 0.02$), progesterone pulse amplitude ($r = -0.85$, $p = 0.007$) on Day 11 and with the progesterone pulse frequency on Day 16 ($r = -0.90$, $p = 0.04$) (I).
In gilts of Study I, the number of embryos (14.0 ± 4.75) (mean ± SD) on Day 22–30 (days of autopsy) correlated negatively with progesterone pulse frequency ($r = -0.84$, $p = 0.04$) and positively with progesterone pulse amplitude ($r = 0.93$, $p = 0.02$) on Day 21.
We found no correlation between LH and numbers of embryos or numbers of corpora lutea in any of the studies.

7

Discussion

We investigated the relationship between vcc progesterone and LH in early pregnancy (Studies I, II and III) and 11 days after insemination (Study I) in the sow in three different studies. We therefore explored the release pattern of these hormones on Days 11 (I), 14 (III), 16 (I, II) and 21 (I, II).

The results of Studies I and II demonstrated that there was no relationship between LH pulsatility and progesterone pulsatility in gilts during early pregnancy. Neither Brussow et al. (2011) found a relationship between these two hormones on Days 13, 15 and 17, nor Langendijk et al. (2017) on Day 11 of pregnancy in gilts. Virolainen et al. (2005) found a relationship in one gilt on Day 22 of pregnancy. Our Studies I and II showed that progesterone pulsatile secretion was independent of LH pulsatile stimulus. In Study I, this was demonstrated on Day 11, when LH pulse frequency and amplitude tended to differ but progesterone pulsatile secretion was similar in pregnant and non-pregnant gilts. The results of Study II underlined this independence by demonstrating that progesterone secretion was pulsatile even in the absence of LH pulses on Day 16 and Day 21.

Day 16 (II) results are comparable with the results of Brussow et al. (2008) during oestrus cycle in Mangaliza sows, which seem to have had a very low LH secretion with no pulses (Brussow et al., 2008). The absence of LH pulses in Mangaliza sows did not affect progesterone pulsatility at Day 12 and Day 15 of the oestrus cycle. Brussow et al. (2008) suggested that the absence of LH pulses might be due to the higher progesterone concentration (of unknown origin) in this breed that seems to feedback strongly on LH release. Similarly to the naturally absent LH pulses in Mangaliza sows, LH pulsatility ceased in Study II by the use of a GnRH agonist. The absence of LH pulses did not change pulsatile secretion pattern of the CL on Day 16 and Day 21. The capacity of the CL to function (Day 16 to Day 21 in Study II) during early pregnancy, in that they secrete progesterone without episodic LH stimulus, was demonstrated by Peltoniemi et al. (1995). Study II, however, showed that this is true also for the pulsatile release of progesterone. Peltoniemi et al. (1995) demonstrated that it took over two weeks after inserting the GnRH agonist implant on Days 14, 21 or 29 until abortion occurred. Study II confirmed that finding and showed that pregnancy was maintained at

least for eight days after cessation of LH pulsatility and thirteen days after insertion of the implant. This result differs from that of Anderson et al. (1967), who demonstrated that CL are dependent on LH support. They defined the first day of the oestrus cycle as the first day of pregnancy and surgically disconnected the hypothalamus from the pituitary gland (stalk-section) of mated gilts on Day 2. Corpora lutea and pregnancy were maintained until Day 16, but regressed and failed by Day 20. Progesterone concentration declined accordingly. The down-regulation of LH in Study II started only after Day 11 and the CL were exposed to LH for a longer time than in the study of Anderson et al. (1967). In addition the stalk section as done by Anderson et al. (1967) cuts off LH release into the circulation completely. In Study II, some LH was present and it might be that a low concentration of LH is sufficient for the corpora lutea to maintain their function.

Although we did not establish a direct relationship between vcc progesterone and LH in gilts, we found that the episodic release patterns of LH and progesterone alternated in some animals, indicating a degree of synchrony. This happened on Day 21 in Study I in four of the six gilts. In these animals, release of the one hormone was pulsatile while the release of the other hormone was basal. It might be that the CL graviditatum, with growing dependency on LH (Anderson et al. 1967), also become more susceptible to LH's episodic stimulus in terms of their own progesterone release.
These results in gilts differed from those of the primiparous sows in Study III, as in the primiparous sows, on Day 14 of pregnancy, as many as 60% of the LH pulses were followed by a progesterone pulse within one hour. No other studies are available on the LH and progesterone relationship in primiparous sows and it is not known whether these results are reproducible. It is unclear why in our primiparous sows, LH pulses would trigger progesterone pulses and in our gilts this did not happen. It is tempting to speculate that during the maturing process (from gilt to primiparous sow), endocrinological cycles mature and start to interact with each other. This could explain why LH pulses are followed by progesterone pulses in sows but not in gilts. However, a CL is formed newly at each ovulation. Therefore, the CL itself may have the same age and grade of maturity on Day 14 in a gilt as in a primiparous sow. Furthermore, luteal mass differs between gilts and multiparous sows (Langendijk and Peltoniemi, 2013). Thus, the major factor determining total luteal mass may be ovulation rate (Willis et al., 2003; Athorn et al., 2012). Additionally, systemic progesterone concentration roughly follows

the development in luteal tissue mass (Tast et al., 2002; Bouwman et al., 2012). The progesterone level is related to the number of CL and pigs with high numbers of ovulation seem to be exposed to high progesterone levels (Guthrie et al., 1974; Knox et al., 2003). Ovulation rate significantly increased with parity (DaSilva, 2016) and the primiparous sows of Study III had more CL (mean 22.5) than the gilts of Studies I (mean 16.25) and II (mean 17.05). Whether this higher number of CL is explanatory for the indicated relationship between LH pulsatility and progesterone pulsatility in Study III remains unclear. In commercial herds, approximately 10% of inseminated sows return to oestrus (Koketsu et al., 2003; Peltoniemi and Kemp, 2019). Oestrus in these sows appears either at a regular (18-24 days), irregular (25-38 days) or late return (39 days or later) interval (Koketsu et al., 2003). Gilts have more regular returns than sows, and sows have more irregular returns than gilts. The regular returns indicate either no conception or failure of maternal recognition. In contrast, irregular returns imply successful conception but subsequently early pregnancy loss (Tast et al., 2002) and late returns suggest late pregnancy loss (Koketsu, 2003). It could be that a higher response rate of the progesterone secretion of corpora lutea to an LH stimulus as seen in Study III compared to the gilts in Study I improves embryonal survival and therefore sows are less likely to show regular returns to oestrus than gilts.

Although the CL is formed newly at every ovulation, the degree of maturity of other organs along the HPG axis probably increases with parity. Studies focusing on the development of the HPG axis from first parity onwards in sows are not available. It could be that a more mature hypothalamus and pituitary gland might react more precisely to progesterone feedback. On the other hand, there are no studies on the quality and quantity of progesterone feedback on the hypothalamus and pituitary gland and it is unclear whether progesterone pulses reach the brain and can be a trigger for LH pulses. However, as shown by the present data, higher ovulation rate results in higher total CL mass and a corresponding higher basal level of metabolized progesterone in sows compared with gilts. Furthermore, a more mature secretory pattern of leptin is likely to play a role as well as maturation of the hypothalamus and the pituitary gland (Barb et al., 2004). Therefore, it is likely that the cue to understand the relationship in Study III between progesterone pulsatility and LH episodic secretion lies in the maturation process of the hypothalamus and/or pituitary gland.

Our research in Studies I, II and III on the relationship between progesterone and LH indicate a difference between gilts and sows. It seems that the association between LH and progesterone in sows is partly direct, in that a progesterone pulse follows an LH pulse, as seen in Study III. Alternatively, it might be partly related to activity in that an active, pulsatile phase of the one hormone follows after an active pulsatile phase of the other hormone in gilts, as in Study I. On the other hand, for gilts, Study II indicates that there would be no relationship or association between these hormones.
Metabolization of progesterone could be demonstrated in Study III. Concentrations in the vena cava caudalis (before entering the liver) were higher and considerably more variation was found than in the vena jugularis (after passage through the liver). A significant difference in the progesterone concentration between these two sampling sites has been reported during the first week (Athorn et al., 2013), during the second and third weeks (Brussow et al., 2011) and during the fourth week (Virolainen et al., 2005) of pregnancy in gilts. Metabolized mean progesterone was lower than the basal and mean concentration before metabolization in Study III (Table 4). The metabolization process levelled out 10 fold differences (between basal and amplitude) in progesterone concentration found in the vena cava caudalis. Our Study III therefore confirms earlier findings that metabolization of progesterone in the liver seems to be dose independent in that it maintains progesterone homeostasis. High progesterone concentrations (for example, during the luteal phase of the cycle (Brussow et al., 2008)) however seem to exert a feedback effect on the HPG axis. It might be that the liver reacts to a longer period of elevated progesterone by adjusting the metabolization rate to increase the post-metabolization concentration. It is unclear for how long progesterone needs to be elevated or if indeed elevated progesterone is the trigger for a higher post-metabolization progesterone concentration. It also needs to be emphasized that progesterone concentration in the vena jugularis is not similar to liver-metabolized progesterone concentration. Since after the metabolization progesterone passes the heart and enters the pulmonary circulation whereafter via the arterial bloodstream it is transported to the brain and only then by venous drainage ends up in the vena jugularis.

Studies I and II (control group) demonstrated that the decline of progesterone that was reported previously for systemic concentrations between Days 8 and 27 (King and Rajamahendran, 1988) and from Day 12 to Day 16 of pregnancy (Pharazyn et al., 1991) was also true

for vena cava caudalis progesterone concentrations. We showed that a decreasing progesterone pulse frequency and amplitude were the reason for the decline in mean progesterone and basal progesterone. To our knowledge, there are no studies comparing pre- and post-uterine progesterone pulses during pregnancy. It remains therefore unclear if the decline in amplitude and pulsatility is partly due to elevated embryonic consumption or if the CLs' secretory capacity simply levels out.
A clear decline in LH concentration from Day 11 to Day 21 of pregnancy was not observed in Studies I and II. A decline in LH receptors has been documented from the follicular phase to the luteal phase (Phoophitphong et al., 2017) and in the luteal phase the number of LH receptors reached the maximum between Days 8 and 10 (Gebarowska et al., 1997). These results demonstrate that the number of LH receptors is dynamic. Though no data is available on LH receptor density during the third and fourth week of pregnancy, it might be that part of the decline in progesterone secretion is a consequence of LH receptor down-regulation. On the other hand, Study II demonstrates that the amplitude and frequency of progesterone pulses declined in both the GnRHa group and control group, questioning if LH or LH receptors have any influence at all. Moreover, progesterone parameters were higher in gilts of the GnRHa group (Study II) than in the gilts of the control group on Days 16 and 21, possibly suggesting that LH was not needed at all for the short-term progesterone release, or that GnRH triggered directly ovarian progesterone release or that absence of LH might even enhance progesterone production and release for some time.
Diurnal rhythmicity was observed in LH release in Study I. The gilts had either two or three LH pulses during twelve hours and these pulses appeared in a specific time frame. Furthermore, control gilts of Study II all had a two-pulse LH rhythm on Day 16. Studies on the timing of LH pulses in sows within the day have so far concentrated on the LH surge before ovulation. The preovulatory LH surge appeared at a specific time of the day in rats (Colombo et al., 1974; Mahoney et al., 2004), which was related to the light-dark cycle (Colombo et al., 1974). Apparently, ovulatory cycles require interactions between the circadian and reproductive systems. Under the management conditions of our gilts, the light- dark cycle and feeding time (Aschoff, 1966) might be external pacemakers for circadian rhythms and activity and sleeping time (Bubenik et al., 2002). The LH synchronicity pattern seen in our gilts seems to show that the hypothalamic circadian clock coordinates tLH secretion within during early pregnancy.

8

Conclusions

I Progesterone secretion pattern is not responsive to LH pulsatile stimulus in that a progesterone pulse would follow an LH pulse within one hour on Day 11, Day 16 and Day 21 of pregnancy in the gilt. Temporal relationships however exist and they appear at the level of an individual.

II Sows seem to differ from gilts in that a temporal relationship between LH and progesterone was demonstrated on Day 14 of pregnancy, where over half of the LH pulses were followed by a progesterone pulse within one hour. This might partly be due to the sexual maturation process.

III Pregnant gilts' pulsatile progesterone release is not dependent on LH pulsatility on Day 16 and Day 21 of pregnancy in gilts. The down-regulation of LH secretion to basal release does not modify pulsatile progesterone secretion.

IV The nature of the pulsatile secretion of progesterone from the CL remains unclear. What is the mechanism behind the pulsatile progesterone secretion and why is progesterone secreted in a pulsatile manner?

Implications and further research

The results of the Study I, II and III regarding LH support of CLs' progesterone release were incongruent. Researches addressing this question in vivo are rare and have been conducted partly in the middle of the last century. Therefore it is important to first clarify the nature and period of the porcine CL dependence on LH.

Further research is needed to clarify whether the LH and vcc progesterone association of the pregnant multiparous sows of Study III are reproduceable, and to establish to what extent parity affects the association. Preferably, this should be studied in multiple phases of the embryonic phase of pregnancy. These studies might clarify if differences in the capacity of the CL to react to LH and adjust their progesterone

secretion contribute to differences in return rates between gilts and sows.
We did not find any further studies concentrating on the CLs' secretory capacity during the period from the rescue of the CL to the completed attachment in vivo. The reason for the decreasing vcc progesterone concentration in this period remains unclear and needs further exploration.

Progesterone concentration was higher in the GnRHa gilts of Study II than in the control group. We discussed the possible mechanisms for this finding in the respective article. However, the influence of any GnRH agonist on ovulation during the luteal phase and its consequent possibility to increase progesterone concentration and its further implication of commercial usage would be worth researching.

9

References

Akinlosotu, B. A., Diehl, J. R., & Gimenez, T. (1988). Prostaglandin E2 counteracts the effects of PGF2 alpha in indomethacin treated cycling gilts. Prostaglandins, 35(1), 81-93. doi:0090-6980(88)90276-6

Anderson, L. L., Dyck, G. W., Mori, H., Henricks, D. M., & Melampy, R. M. (1967). Ovarian function in pigs following hypophysial stalk transection or hypophysectomy. American Journal of Physiology, 212(5), 1188-1194

Anderson, L. L., Butcher, R. L., & Melampy, R. M. (1961). Subtotal hysterectomy and ovarian function in gilts. Endocrinology, 69, 571-580. doi:10.1210/endo-69-3-571

Aschoff, J. (1966). Circadian activity pattern with two peaks. Ecology, 47(4), 657-662. doi:10.2307/1933949

Ashworth, C. J. (1991). Effect of pre-mating nutritional status and post-mating progesterone supplementation on embryo survival and conceptus growth in gilts. Animal Reproduction Science, 26(3-4), 311-321

Ashworth, C. J., Antipatis, C., & Beattie, L. (1999). Effects of pre- and post-mating nutritional status on hepatic function, progesterone concentration, uterine protein secretion and embryo survival in meishan pigs. Reproduction, Fertility and Development, 11(1), 67-73. doi:10.1071/RD99007

Athorn, R. Z., Stott, P., Bouwman, E. G., Ashman, R., O'Leary, S., Nottle, M., & Langendijk, P. (2011). Direct ovarian-uterine transfer of progesterone increases embryo survival in gilts. Reproduction, Fertility, and Development, 23(7), 921-928. doi:10.1071/RD10333

Athorn, R., Stott, P., Bouwman, E., Chen, T. Y., Kennaway, D., & Langendijk, P. (2013). Effect of feeding level on luteal function and progesterone concentration in the vena cava during early pregnancy in gilts. Reproduction, Fertility, and Development, 25, 531-8. doi:10.1071/RD11295

Athorn, R., Stott, P., Bouwman, E., Edwards, A., Blackberry, M., Martin, G., & Langendijk, P. (2013). Feeding level and dietary energy source have no effect on embryo survival in gilts, despite changes in systemic progesterone levels. Animal Production Science, 53, 30-37. doi:10.1071/AN12004

Baerwald, A. R., Adams, G. P., & Pierson, R. A. (2005). Form and function of the corpus luteum during the human menstrual cycle. Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology, 25(5), 498-507. doi:10.1002/uog.1891

Balet, L., Janett, F., Husler, J., Piechotta, M., Howard, R., Amatayakul-Chantler, S., . . . Hirsbrunner, G. (2014). Immunization against gonadotropin-releasing hormone in dairy cattle: Antibody titers, ovarian function, hormonal levels, and reversibility. Journal of Dairy Science, 97(4), 2193-2203. doi:10.3168/jds.2013-7602

Barb, C. R., Hausman, G. J., & Czaja, K. (2005). Leptin: A metabolic signal affecting central regulation of reproduction in the pig. Domestic Animal Endocrinology, 29(1), 186-192. doi: 10.1016/j.domaniend.2005.02.024

Barb, C. R., Hausman, G. J., & Lents, C. A. (2008). Energy metabolism and leptin: Effects on neuroendocrine regulation of reproduction in the gilt and sow. Reproduction in Domestic Animals, 43, 324-330. doi:10.1111/j.1439-0531.2008.01173.x

Barb, C. R., & Kraeling, R. R. (2004). Role of leptin in the regulation of gonadotropin secretion in farm animals. Animal Reproduction Science, 82-83, 155-167. doi:10.1016/j.anireprosci.2004.04.032

Barb, C. R., Kraeling, R. R., & Rampacek, G. B. (2001). Nutritional regulators of the hypothalamic-pituitary axis in pigs. Reproduction (Cambridge, England) Supplement, 58, 1-15

Bazer, F. W., & Thatcher, W. W. (1977). Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F2alpha by the uterine endometrium. Prostaglandins, 14(2), 397-400. doi:0090-6980(77)90185-X

Benoit, A. M., & Dailey, R. A. (1991). Catheterization of the caudal vena cava via the lateral saphenous vein in the ewe, cow, and gilt: An alternative to utero-ovarian and medial coccygeal vein catheters. Journal of Animal Science, 69(7), 2971-2979

Bienstock, J., Fox, H., & Wallach, E. (2015). In Bienstock J., Fox H. and Wallach E. (Eds.), Johns Hopkins Manual of Gynecology and Obstetrics (5th ed.). Philadelphia, USA: Lippincott Williams and Wilkins

Blitek, A., & Ziecik, A. J. (2005). Effect of LH on prostaglandin F2alpha and prostaglandin E2 secretion by cultured porcine endometrial cells. Reproduction (Cambridge, England), 130(1), 105-112. doi:130/1/105

Brady, P. C., Kaser, D. J., Ginsburg, E. S., Ashby, R. K., Missmer, S. A., Correia, K. F., & Racowsky, C. (2014). Serum progesterone concentration on day of embryo transfer in donor oocyte cycles. Journal of Assisted Reproduction and Genetics, 31(5), 569-575. doi:10.1007/s10815-014-0199-y

Brandt, Y., Lundeheim, N., Madej, A., Rodriguez-Martinez, H., & Einarsson, S. (2007). Effects of ACTH injections during estrus on concentrations and patterns of progesterone, oestradiol, LH, and inhibin α and time of ovulation in the sow. Domestic Animal Endocrinology, 32(2), 122-137

Brandt, Y., Madej, A., Rodriguez-Martinez, H., & Einarsson, S. (2007). Effects of exogenous ACTH during oestrus on early embryo development and oviductal transport in the sow. Reproduction in Domestic Animals, 42(2), 118-125

Brussow, K. P., Schneider, F., Tuchscherer, A., Ratky, J., Kraeling, R. R., & Kanitz, W. (2007). Luteinizing hormone release after administration of the gonadotropin-releasing hormone agonist fertilan (goserelin) for synchronization of ovulation in pigs. Journal of Animal Science, 85(1), 129-137. doi:10.2527/jas.2006-281

Brussow, K. P., Schneider, F., Wollenhaupt, K., & Tuchscherer, A. (2011). Endocrine effects of GnRH agonist application to early pregnant gilts. Journal of Reproduction and Development, 57(2), 242-248. doi:JST.JSTAGE/jrd/10-021O

Brussow, K., Schneider, F., Tuchscherer, A., Egerszegi, I., & Ratky, J. (2008). Comparison of luteinizing hormone, leptin and progesterone levels in the systemic circulation (vena jugularis) and near the ovarian circulation (vena cava caudalis) during the oestrous cycle in mangalica and landrace gilts. Journal of Reproduction and Development, 54(6), 431- 438. doi:10.1262/jrd.20069

Bubenik, G. A., Pang, S. F., Cockshut, J. R., Smith, P. S., Grovum, L. W., Friendship, R. M., & Hacker, R. R. (2000). Circadian variation of portal, arterial and venous blood levels of melatonin in pigs and its relationship to food intake and sleep. Journal of Pineal Research, 28(1), 9-15. doi:10.1034/j.1600-079x.2000.280102.x

Buhi, W. C., Alvarez, I. M., Sudhipong, V., & Dones-Smith, M. M. (1990). Identification and characterization of de novo-synthesized porcine oviductal secretory proteins. Biology of Reproduction, 43(6), 929-938. doi:10.1095/biolreprod43.6.929

Carnahan, K. G., Prince, B. C., & Mirando, M. A. (1996). Exogenous oxytocin stimulates uterine secretion of prostaglandin F2 alpha in cyclic and early pregnant swine. Biology of Reproduction, 55(4), 838-843. doi:10.1095/biolreprod55.4.838

Cassar, G., Chapeau, C., & King, G. J. (1994). Effects of increased dietary energy after mating on developmental uniformity and survival of porcine conceptuses. Journal of Animal Science, 72(5), 1320-1324. doi:10.2527/1994.7251320x

Chang, J., Frandsen, S., & Gadsby, J. E. (2017). Prostaglandin synthesis by the porcine corpus luteum: Effect of tumor necrosis factor-α. Domestic Animal Endocrinology, 58, 53-62. doi: 10.1016/j.domaniend.2016.07.001

Chen, T. Y., Stott, P., Athorn, R. Z., Bouwman, E. G., & Langendijk, P. (2012). Undernutrition during early follicle development has irreversible effects on ovulation rate and embryos. Reproduction, Fertility, and Development, 24(6), 886-892. doi:10.1071/RD11292

Christenson, L. K., Anderson, L. H., Ford, S. P., & Farley, D. B. (1994). Luteal maintenance during early pregnancy in the pig: Role for prostaglandin E2. Prostaglandins, 47(1), 61- 75. doi: 10.1016/0090-6980(94)90075-2

Claus, R., & Weiler, U. (1985). Influence of light and photoperiodicity on pig prolificacy. Journal of Reproduction and Fertility Supplement, 33, 199-208

Clawitter, J., Trout, W. E., Burke, M. G., Araghi, S., & Roberts, R. M. (1990). A novel family of progesterone-induced, retinol-binding proteins from uterine secretions of the pig. The Journal of Biological Chemistry, 265(6), 3248-3255

Clay, A. L., Neely, L. H., C, R. B., & J, J. F. (2008). Central and peripheral administration of kisspeptin activates gonadotropin but not somatotropin secretion in prepubertal gilts. Reproduction, 135(6), 879-887. doi:10.1530/REP-07-0502

Colombo, J. A., Baldwin, D. M., & Sawyer, C. H. (1974). Timing of the estrogen-induced release of LH in ovariectomized rats under an altered lighting schedule. Proceedings of the Society for Experimental Biology and Medicine.Society for Experimental Biology and Medicine (New York, N.Y.), 145(3), 1125-1127 doi:10.3181/00379727-145-37965

Constantin, S. (2017). Progress and challenges in the search for the mechanisms of pulsatile gonadotropin-releasing hormone secretion. Frontiers in Endocrinology, 8, 180. doi:10.3389/fendo.2017.00180

Corner Jr, G. W. (1956). The histological dating of the human corpus luteum of menstruation. American Journal of Anatomy, 98(3), 377-401

Cosgrove, J. R., Booth, P. J., & Foxcroft, G. R. (1991). Opioidergic control of gonadotrophin secretion in the prepubertal gilt during restricted feeding and realimentation. Journal of Reproduction and Fertility, 91(1), 277-284. doi:10.1530/jrf.0.0910277

Cran, D. G., Osborn, J. C., & Rushton, D. (1983). Thecal vasculature and oocyte maturation during follicular atresia in the sheep and pig. Reproduction, Nutrition, Developement, 23(2a), 285-292. doi:10.1051/rnd:19830212

Da Silva, C. L. A., van den Brand, H., Laurenssen, B. F. A., Broekhuijse, M. L. W. J., Knol, E. F., Kemp, B., & Soede, N. M. (2016). Relationships between ovulation rate and embryonic and placental characteristics in multiparous sows at 35 days of pregnancy. Animal, 10(7), 1192-1199. doi:10.1017/S175173111600015X

Dhindsa, D. S., & Dziuk, P. J. (1968). Effect on pregnancy in the pig after killing embryos or fetuses in one uterine horn in early gestation. Journal of Animal Science, 27(1), 122-126

Dobson, H., & Smith, R. F. (2000). What is stress, and how does it affect reproduction? Animal Reproduction Science, 60-61, 743-752. doi://doi.org/10.1016/S0378-4320(00)00080-4

Dziuk, P. (1985). Effect of migration, distribution and spacing of pig embryos on pregnancy and fetal survival. Journal of Reproduction and Fertility. Supplement, 33, 57-63

Ebling, F. J. P., Maywood, E. S., Humby, T., & Hastings, M. H. (1992). Circadian and photoperiodic time measurement in male syrian hamsters following lesions of the melatonin-binding sites of the paraventricular thalamus. Journal of Biological Rhythms, 7(3), 241-254. doi:10.1177/074873049200700305

Eiler, H. (1978). Effect of luteinizing hormone on ovarian release of steroids: Ovarian vein- sampling technique in conscious gilt. American Journal of Veterinary Research, 39(10), 1695-1698

Einarsson, S., Sjunnesson, Y., Hulten, F., Eliasson-Selling, L., Dalin, A. M., Lundeheim, N., & Magnusson, U. (2014). A 25 years experience of group-housed sows-reproduction in animal welfare-friendly systems. Acta Veterinaria Scandinavica, 56, 37-37. doi:10.1186/1751-0147-56-37

Farin, C. E., Moeller, C. L., Sawyer, H. R., Gamboni, F., & Niswender, G. D. (1986). Morphometric analysis of cell types in the ovine corpus luteum throughout the estrous cycle. Biology of Reproduction, 35(5), 1299-1308. doi:10.1095/biolreprod35.5.1299

Filicori, M., Butler, J. P., & Crowley, W. F.,Jr. (1984). Neuroendocrine regulation of the corpus luteum in the human. evidence for pulsatile progesterone secretion. The Journal of Clinical Investigation, 73(6), 1638-1647. doi:10.1172/JCI111370

Fischer, H. E., Bazer, F. W., & Fields, M. J. (1985). Steroid metabolism by endometrial and conceptus tissues during early pregnancy and pseudopregnancy in gilts. Journal of Reproduction and Fertility, 75(1), 69-78. doi:10.1530/jrf.0.0750069

Flint, A. P., & Sheldrick, E. L. (1982). Ovarian secretion of oxytocin is stimulated by prostaglandin. Nature, 297(5867), 587-588. doi:10.1038/297587a0

Foote, A. P., Freking, B. A., Nonneman, D. J., Berry, E. D., Wright, E. C., Thorson, J. F., . . . Clapper, J. A. (2018). Energy balance affects pulsatile secretion of luteinizing hormone from the adenohypophesis and expression of neurokinin B in the hypothalamus of ovariectomized gilts†. Biology of Reproduction, 99(2), 433-445. doi:10.1093/biolre/ioy069

Franczak, A., & Kotwica, G. (2010). Androgens and estradiol-17β production by porcine uterine cells: In vitro study. Theriogenology, 73(2), 232-241. doi: 10.1016/j.theriogenology.2009.09.004

Franczak, A., Kotwica, G., Kurowicka, B., Oponowicz, A., Woclawek-Potocka, I., & Petroff, B. K. (2006). Expression of enzymes of cyclooxygenase pathway and secretion of prostaglandin E2 and F2alpha by porcine myometrium during luteolysis and early pregnancy. Theriogenology, 66(5), 1049-1056. doi:S0093-691X(06)00156-7

Frank, M., Bazer, F. W., Thatcher, W. W., & Wilcox, C. J. (1977). A study of prostaglandin F2alpha as the luteolysin in swine: III effects of estradiol valerate on prostaglandin F, progestins, estrone and estradiol concentrations in the utero-ovarian vein of nonpregnant gilts. Prostaglandins, 14(6), 1183-1196. doi:0090-6980(77)90295-7

Freick, M., Weber, O., Passarge, O., & Neubert, T. (2014). Use of gonadorelin[6-D-phe] at day 0 or 12 after insemination to increase the conception rate in a large dairy herd in saxony/germany. [Einsatz von Gonadorelin [6-D-Phe] am Tag 0 oder 12 post inseminationem zur Steigerung der Konzeptionsrate in einer sachsischen Milchviehgrossanlage] Tierarztliche Praxis. Ausgabe G, Grosstiere/Nutztiere, 42(6), 331- 342. doi:10.15653/TPG-140315

Fritz, M. A., & Fitz, T. A. (1991). The functional microscopic anatomy of the corpus luteum: The “small cell”-”large cell” controversy. Clinical Obstetrics and Gynecology, 34(1), 144-156. doi:10.1097/00003081-199103000-00017

Gebarowska, D., Ziecik, A. J., & Gregoraszczuk, E. L. (1997). Luteinizing hormone receptors on granulosa cells from preovulatory follicles and luteal cells throughout the oestrous cycle of pigs. Animal Reproduction Science, 49(2-3), 191-205. doi:S0378-4320(97)00060-2

Geisert, R. D., Renegar, R. H., Thatcher, W. W., Roberts, R. M., & Bazer, F. W. (1982). Establishment of pregnancy in the pig: I. interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biology of Reproduction, 27(4), 925-939. doi:10.1095/biolreprod27.4.925

Geisert, R. D., Zavy, M. T., Wettemann, R. P., & Biggers, B. G. (1987). Length of pseudopregnancy and pattern of uterine protein release as influenced by time and duration of oestrogen administration in the pig. Reproduction, 79(1), 163-172. doi:10.1530/jrf.0.0790163

Gill, J. L., & Hafs, H. D. (1971). Analysis of repeated measurements of animals. Journal of Animal Science, 33(2), 331-336

Gross, T. S., Mirando, M. A., Young, K. H., Beers, S., Bazer, F. W., & Thatcher, W. W. (1990). Reorientation of prostaglandin F secretion by calcium ionophore, estradiol, and prolactin in perifused porcine endometrium*. Endocrinology, 127(2), 637-642. doi:10.1210/endo-127-2-637

Guthrie, H. D., Henricks, D. M., & Handlin, D. L. (1974). Plasma hormone levels and fertility in pigs induced to superovulate with PMSG. Journal of Reproduction and Fertility, 41(2), 361-370. doi:10.1530/jrf.0.0410361

Han, X., Zhou, Y., Zeng, Y., Sui, F., Liu, Y., Tan, Y., . . . Zeng, X. (2017). Effects of active immunization against GnRH versus surgical castration on hypothalamic-pituitary function in boars. Theriogenology, 97, 89-97. doi: 10.1016/j.theriogenology.2017.04.038

Hehnke, K. E., Christenson, L. K., Ford, S. P., & Taylor, M. (1994). Macrophage infiltration into the porcine corpus luteum during prostaglandin F2 alpha-induced luteolysis. Biology of Reproduction, 50(1), 10-15. doi:10.1095/biolreprod50.1.10

Heinonen, M. L., Raekallio, M. R., Oliviero, C., Ahokas, S., & Peltoniemi, O. A. (2009). Comparison of azaperone-detomidine-butorphanol-ketamine and azaperone-tiletamine-zolazepam for anaesthesia in piglets. Veterinary Anaesthesia and Analgesia, 36(2), 151-157. doi:10.1111/j.1467-2995.2008.00443.x

Hiney, J. K., Pine, M. D., Srivastava, V. K., & Dees, W. L. (2009). Insulin-like growth factor-I activates KiSS-1 gene expression in the brain of the prepubertal female rat. Endocrinology, 150(1), 376-384. doi:10.1210/en.2008-0954

Holzbauer, M., & Newport, H. M. (1969). Adrenal secretion rates and adrenal tissue concentrations of pregnenolone, progesterone, 11 beta OH-androstenedione and some other steroids in young pigs and dogs. The Journal of Physiology, 200(3), 821-848. doi:10.1113/jphysiol.1969.sp008724

Hoving, L. L., Soede, N. M., Feitsma, H., & Kemp, B. (2012a). Embryo survival, progesterone profiles and metabolic responses to an increased feeding level during second gestation in sows. Theriogenology, 77(8), 1557-1569. doi:10.1016/j.theriogenology.2011.11.024

Hoving, L. L., Soede, N. M., Feitsma, H., & Kemp, B. (2012b). Lactation weight loss in primiparous sows: Consequences for embryo survival and progesterone and relations with metabolic profiles. Reproduction in Domestic Animals, 47(6), 1009-1016. doi:10.1111/j.1439-0531.2012.02007.x

Irwig, M. S., Fraley, G. S., Smith, J. T., Acohido, B. V., Popa, S. M., Cunningham, M. J., . . . Steiner, R. A. (2004). Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology, 80(4), 264-272. doi:10.1159/000083140

Iwai, M., Yasuda, K., Fukuoka, M., Iwai, T., Takakura, K., Taii, S., . . . Mori, T. (1991). Luteinizing hormone induces progesterone receptor gene expression in cultured porcine granulosa cells. Endocrinology, 129(3), 1621-1627. doi:10.1210/endo-129-3-1621

Janett, F., Stump, R., Burger, D., & Thun, R. (2009). Suppression of testicular function and sexual behavior by vaccination against GnRH (equity) in the adult stallion. Animal Reproduction Science, 115(1-4), 88-102. doi:10.1016/j.anireprosci.2008.11.011

Jarry, H., Einspanier, A., Kanngiesser, L., Dietrich, M., Pitzel, L., Holtz, W., & Wuttke, W. (1990). Release and effects of oxytocin on estradiol and progesterone secretion in porcine corpora lutea as measured by an in vivo microdialysis system. Endocrinology, 126(5), 2350

Junaidi, A., Williamson, P. E., Martin, G. B., Stanton, P. G., Blackberry, M. A., Cummins, J. M., & Trigg, T. E. (2007). Pituitary and testicular endocrine responses to exogenous gonadotrophin-releasing hormone (GnRH) and luteinising hormone in male dogs treated with GnRH agonist implants. Reproduction, Fertility and Development, 19(8), 891-898. doi: 10.1071/RD07088

Junko Tomikawa, Tamami Homma, Shigeyuki Tajima, Takako Shibata, Yoko Inamoto, Kenji Takase, . . . Hiroko Tsukamura. (9863). Molecular characterization and estrogen regulation of hypothalamic KISS1 gene in the pig[1]. Biology of Reproduction, 82(2), 313-319. doi: 10.1095/biolreprod.109.079863

Karaconji, B., Lloyd, B., Campbell, N., Meaney, D., & Ahern, T. (2015). Effect of an anti-gonadotropin-releasing factor vaccine on sexual and aggressive behaviour in male pigs during the finishing period under australian field conditions. Australian Veterinary Journal, 93(4), 121-123. doi:10.1111/avj.12307

Kauffold, J., Rohrmann, H., Boehm, J., & Wehrend, A. (2010). Effects of long-term treatment with the GnrH agonist deslorelin (suprelorin) on sexual function in boars. Theriogenology, 74(5), 733-740. doi:10.1016/j.theriogenology.2010.03.026

Kauffold, J., von dem Bussche, B., Failing, K., & Wehrend, A. (2010). Use of B-mode ultrasound and grey-scale analysis to study uterine echogenicity in the pig. Journal of Reproduction and Development, 56(4), 444-448. doi:10.1262/jrd.09-220T

Kershaw, E. E., & Flier, J. S. (2004). Adipose tissue as an endocrine organ. The Journal of Clinical Endocrinology & Metabolism, 89(6), 2548-2556. doi:10.1210/jc.2004-0395

King, G. J., & Rajamahendran, R. (1988). Comparison of plasma progesterone profiles in cyclic, pregnant, pseudopregnant and hysterectomized pigs between 8 and 27 days after oestrus. Journal of Endocrinology, 119(1), 111-116

King, G. J., & Ackerley, C. A. (1985). Demonstration of oestrogens in developing pig trophectoderm and yolk sac endoderm between days 10 and 16. Journal of Reproduction and Fertility, 73(2), 361-367. doi:10.1530/jrf.0.0730361

Koketsu Y (2003). Re-serviced females on commercial swine breeding farms. Journal of Veterinary Medical Science, 65(12), 1287-1291. doi:10.1292/jvms.65.1287

Knapczyk, K., Duda, M., Durlej, M., Galas, J., Koziorowski, M., & Slomczynska, M. (2008). Expression of estrogen receptor α (ERα) and estrogen receptor β (ERβ) in the ovarian follicles and corpora lutea. Domestic Animal Endocrinology, 35 (2), 170-179. doi: 10.1016/j.domaniend.2008.05.001

Knight, J. W., Bazer, F. W., Thatcher, W. W., Franke, D. E., & Wallace, H. D. (1977). Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelations among hormonal status, placental development, fetal fluids and fetal growth. Journal of Animal Science, 44(4), 620-637. doi:10.2527/jas1977.444620x

Knox, R. V., Naber, C. H., & Zimmerman, D. R. (1991). Follicle-stimulating hormone (FSH) during the secondary surge in gilts as influenced by administration of porcine follicular fluid (pFF). Journal of Animal Science, 69(2), 761-769. doi:10.2527/1991.692761x

Knox, R. V., Vatzias, G., Naber, C. H., & Zimmerman, D. R. (2003). Plasma gonadotropins and ovarian hormones during the estrous cycle in high compared to low ovulation rate gilts. Journal of Animal Science, 81(1), 249-260. doi:10.2527/2003.811249x

Kraeling, R. R., & Davis, B. J. (1974). Termination of pregnancy by hypophysectomy in the pig. Journal of Reproduction and Fertility, 36(1), 215-217. doi:10.1530/jrf.0.0360215

Krzymowski, T., Czarnocki, J., Koziorowski, M., Kotwica, J., & Stefańczyk-Krzymowska, S. (1986). Counter current transfer of 3H-PGF2α in the mesometrium: A possible mechanism for prevention of luteal regression. Animal Reproduction Science, 11, 259- 272. doi:10.1016/0378-4320(86)90129-6

Kurzynska, A., Bogacki, M., Chojnowska, K., & Bogacka, I. (2014). Peroxisome proliferator activated receptor ligands affect progesterone and 17beta-estradiol secretion by porcine corpus luteum during early pregnancy. Journal of Physiology and Pharmacology : An Official Journal of the Polish Physiological Society, 65(5), 709-717

Langendijk, P., and Peltoniemi, O. (2013). How does nutrition influence luteal function and early embryo survival? In H. Rodrigues-Martinez, N. M. Soede and W. L. Flowers (Ed.), Control of pig reproduction IX (pp. 145–158)

Langendijk, P., Bouwman, E. G., Chen, T. Y., Koopmanschap, R. E., & Soede, N. M. (2017). Temporary undernutrition during early gestation, corpora lutea morphometrics, ovarian progesterone secretion and embryo survival in gilts. Reproduction, Fertility and Development, 29(7), 1349-1355. doi: 10.1071/RD15520

Langendijk, P., Dieleman, S. J., van den Ham, C. M., Hazeleger, W., Soede, N. M., & Kemp, B. (2007). LH pulsatile release patterns, follicular growth and function during repetitive periods of suckling and non-suckling in sows. Theriogenology, 67(5), 1076-1086. doi: 10.1016/j.theriogenology.2006.12.008

Leal, D. F., Muro, B. B. D., Nichi, M., Almond, G. W., Viana, C. H. C., Vioti, G., . . . Garbossa, C. A. P. (2019). Effects of post-insemination energy content of feed on embryonic survival in pigs: A systematic review. Animal Reproduction Science, 205, 70- 77. doi: 10.1016/j.anireprosci.2019.04.005

Lemon, M., & Loir, M. (1977). Steroid release in vitro by two luteal cell types in the corpus luteum of the pregnant sow. The Journal of Endocrinology, 72(3), 351-359. doi:10.1677/joe.0.0720351

Lemon, M., & Mauléon, P. (1982). Interaction between two luteal cell types from the corpus luteum of the sow in progesterone synthesis in vitro. Reproduction, 64(2), 315-323. doi:10.1530/jrf.0.0640315

Leshin, L. S., Kraeling, R. R., Barb, C. R., & Rampacek, G. B. (1992). Associated luteinizing hormone-releasing hormone and luteinizing hormone secretion in ovariectomized gilts. Domestic Animal Endocrinology, 9(1), 77-88. doi: 10.1016/0739-7240(92)90011-L

Love, R. J. (1981). Seasonal infertility in pigs. The Veterinary Record, 109(18), 407-409. Luck, M. R., & Jungclas, B. (1988). The time-course of oxytocin secretion from cultured bovine granulosa cells, stimulated by ascorbate and catecholamines. Journal of Endocrinology, 116(2), 247-258. doi:10.1677/joe.0.1160247

Luck, M. R., & Jungclas, B. (1988). The time-course of oxytocin secretion from cultured
bovine granulosa cells, stimulated by ascorbate and catecholamines. Journal of
Endocrinology, 116(2), 247-258. doi:10.1677/joe.0.1160247

Magness, R. R., Reynolds, L. P., & Ford, S. P. (1986). Evidence for uterine metabolism of progesterone during early pregnancy in the pig. Theriogenology, 25(4), 551-558. Magon, N. (2011). Gonadotropin releasing hormone agonists: Expanding vistas. Indian Journal of Endocrinology and Metabolism, 15(4), 261-267. doi:10.4103/2230-8210.85575

Magon, N. (2011). Gonadotropin releasing hormone agonists: Expanding vistas. Indian Journal of Endocrinology and Metabolism, 15(4), 261-267. doi:10.4103/2230-8210.85575

Mahoney, M. M., Sisk, C., Ross, H. E., & Smale, L. (2004). Circadian regulation of gonadotropin-releasing hormone neurons and the preovulatory surge in luteinizing hormone in the diurnal rodent, arvicanthis niloticus, and in a nocturnal rodent, rattus norvegicus. Biology of Reproduction, 70(4), 1049-1054. doi:10.1095/biolreprod.103.021360

Marques, P., Skorupskaite, K., George, J. T., & Anderson, R. A. (2000). Physiology of GNRH and gonadotropin secretion. In K. R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, K. Dungan, A. Grossman, . . . D. P. Wilson, South Dartmouth (MA): doi:NBK279070

Martyniak, M., Franczak, A., & Kotwica, G. (2018). Synthesis of steroid hormones in the porcine oviduct during early pregnancy. Reproductive Biology 18(2), 143-150. doi:10.1016/j.repbio.2018.02.002

McClellan, M. C., Dieckman, M. A., Abel, J. H.,Jr, & Niswender, G. D. (1975). Luteinizing hormone, progesterone and the morphological development of normal and superovulated corpora lutea in sheep. Cell and Tissue Research, 164(3), 291-307. doi:10.1007/bf00223011

Meduri, G., Vu Hai, M. T., Jolivet, A., Takemori, S., Kominami, S., Driancourt, M. A., & Milgrom, E. (1996). Comparison of cellular distribution of LH receptors and steroidogenic enzymes in the porcine ovary. The Journal of Endocrinology, 148(3), 435- 446. doi:10.1677/joe.0.1480435

Messager, S., Chatzidaki, E. E., Ma, D., Hendrick, A. G., Zahn, D., Dixon, J., . . . Aparicio, Samuel A. J. R. (2005). Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proceedings of the National Academy of Sciences of the United States of America, 102(5), 1761-1766. doi:10.1073/pnas.0409330102

Meyer, A. E., Pfeiffer, C. A., Brooks, K. E., Spate, L. D., Benne, J. A., Cecil, R., . . . Geisert, R. D. (2019). New perspective on conceptus estrogens in maternal recognition and pregnancy establishment in the pig†. Biology of Reproduction, 101(1), 148-161. doi:10.1093/biolre/ioz058

Miller, H. M., Foxcroft, G. R., Squires, J., & Aherne, F. X. (1999). The effects of feed intake and body fatness on progesterone metabolism in ovariectomized gilts. Journal of Animal Science, 77(12), 3253-3261

Moeljono, M. P., Thatcher, W. W., Bazer, F. W., Frank, M., Owens, L. J., & Wilcox, C. J. (1977). A study of prostaglandin F2alpha as the luteolysin in swine: II characterization and comparison of prostaglandin F, estrogens and progestin concentrations in utero-ovarian vein plasma of nonpregnant and pregnant gilts. Prostaglandins, 14(3), 543-555. doi:0090-6980(77)90268-4

Munsterhjelm, C., Valros, A., Heinonen, M., Hälli, O., & Peltoniemi, O. (2008). Housing during early pregnancy affects fertility and behaviour of sows. Reproduction in Domestic Animals, 43(5), 584-591. doi:10.1111/j.1439-0531.2007.00956.x

Murphy, B., Gévry, N., Ruiz Cortés, Z., Coté, F., Downey, B., & Sirois, J. (2001). Formation and early development of the corpus luteum in pigs. Reproduction (Cambridge, England) Supplement, 58, 47-63

Navarro C, S. P. (2012). Pharmacodynamics and pharmacokinetics of a sustained-release implant of deslorelin in companion animals. Proceedings of the 7th International Symposium on Canine and Feline Reproduction Whistler, BC, Canada, 26-29, 177-178

Navarro, V. M., Gottsch, M. L., Chavkin, C., Okamura, H., Clifton, D. K., & Steiner, R. A. (2009). Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. The Journal of Neuroscience, 29(38), 11859-11866. doi:10.1523/JNEUROSCI.1569-09.2009

Niswender, G. D., Juengel, J. L., Silva, P. J., Keith Rollyson, M., & McIntush, E. W. (2000). Mechanisms controlling the function and life span of the corpus luteum. Physiological Reviews, 80(1), 1-29. doi:10.1152/physrev.2000.80.1.1

Norrby, M., Madsen, M., Saravia, F., Lundeheim, N., & Madej, A. (2011). Genistein alters the release of oxytocin, prostaglandins, cortisol and LH during insemination in gilts. Reproduction in Domestic Animals = Zuchthygiene, 46(2), 316-324. doi:10.1111/j.1439-0531.2010.01669.x

Okano, A., Kishi, H., Takahashi, H., & Takahashi, M. (2006). Tumor necrosis factor alpha induces apoptosis in cultured porcine luteal cells. Journal of Reproduction and Development, 52(2), 301-306. doi:10.1262/jrd.17045

Østrup, E., Vejlsted, M., Nielsen, M. B., Madsen, M. T., & Maddox-Hyttel, P. (2008). 107 early embryonic survival in the pig is higher than conventionally reported. Reproduction, Fertility and Development, 21(1), 153-154

Ottobre, J. S., Eyster, K. M., & Stouffer, R. L. (1984). Comparisons of gonadotropin binding sites and progesterone concentrations among the corpora lutea of individual pigs. Biology of Reproduction, 30(2), 271-277. doi:10.1095/biolreprod30.2.271

Parvizi, N., Elsaesser, F., Smidt, D., & Ellendorf, F. (1976). Plasma luteinizing hormone and progesterone in the adult female pig during the oestrous cycle, late pregnancy and lactation, and after ovariectomy and pentobarbitone treatment. Journal of Endocrinology, 69(2), 193-203. doi:10.1677/joe.0.0690193

Pearce, G. P., Paterson, A. M., & Hughes, P. E. (1988). Effect of short-term elevations in plasma cortisol concentration on LH secretion in prepubertal gilts. Journal of Reproduction and Fertility, 83(1), 413-418. doi:10.1530/jrf.0.0830413

Peltoniemi, O. A. T., Easton, B. G., Love, R. J., Klupiec, C., & Evans, G. (1995). Effect of chronic treatment with a GnRH agonist (goserelin) on LH secretion and early pregnancy in gilts. Animal Reproduction Science, 40(1), 121-133. doi:10.1016/0378-4320(95)01400-T

Peltoniemi, O. A., Heinonen, M., Leppavuori, A., & Love, R. J. (1999). Seasonal effects on reproduction in the domestic sow in Finland--a herd record study. Acta Veterinaria Scandinavica, 40(2), 133-144

Peltoniemi, O. A. T., Love, R. J., Klupiec, C., & Evans, G. (1997). Effect of feed restriction and season on LH and prolactin secretion, adrenal response, insulin and FFA in group housed pregnant gilts. Animal Reproduction Science, 49(2), 179-190. doi:10.1016/S0378-4320(97)00062-6

Peltoniemi, O. A., & Virolainen, J. V. (2006). Seasonality of reproduction in gilts and sows. Society of Reproduction and Fertility Supplement, 62, 205-218

Peltoniemi, O. & Kemp, B. (2019). Infertility in the Pig and the Control of Pig Herd Fertility, Veterinary Reproduction and Obstetrics. Noakes, D. E., Parkinson, T. J. & England, G. C. W. (eds.). 10 ed. Netherlands: Elsevier, Vol. 2019. p. 581-592 13p

Pharazyn, A., Foxcroft, G. R., & Aherne, F. X. (1991). Temporal relationship between plasma progesterone concentrations in the utero-ovarian and jugular veins during early pregnancy in the pig. Animal Reproduction Science, 26(3–4), 323-332. doi:10.1016/0378-4320(91)90057-7

Phoophitphong, D., Srisuwatanasagul, S., & Tummaruk, P. (2017). Immunohistochemical localization of luteinizing hormone receptor in the cyclic gilt ovary. Anatomia, Histologia, Embryologia, 46(1), 94-100. doi:10.1111/ahe.12255

Pitzel, L., Jarry, H., & Wuttke, W. (1993). Different steroidogenic response of young and aged porcine small and large luteal cells to prostaglandin F2 alpha, oxytocin and estradiol. Experimental and Clinical Endocrinology, 101(4), 255-261. doi:10.1055/s- 0029-1211241

Polge, C., Rowson, L. E. A., & Chang, M. C. (1966). The effect of reducing the number of embryos during early stages of gestation in the maintenance of pregnancy in the pig. Reproduction, 12(2), 395-397. doi:10.1530/jrf.0.0120395

Pope, W. F. (1988). Uterine asynchrony: A cause of embryonic loss. Biology of Reproduction, 39(5), 999-1003

Prime, G. R., & Symonds, H. W. (1993). Influence of plane of nutrition on portal blood flow and the metabolic clearance rate of progesterone in ovariectomized gilts. Journal of Agricultural Science, Cambridge, 121, 389-397

Prunier, A., & Quesnel, H. (2000). Influence of the nutritional status on ovarian development in female pigs. Animal Reproduction Science, 60-61, 185-197. doi:10.1016/S0378- 4320(00)00093-2

Przygrodzka, E., Witek, K. J., Kaczmarek, M. M., Andronowska, A., & Ziecik, A. J. (2015). Expression of factors associated with apoptosis in the porcine corpus luteum throughout the luteal phase of the estrous cycle and early pregnancy: Their possible involvement in acquisition of luteolytic sensitivity. Theriogenology, 83(4), 535-545. doi:10.1016/j.theriogenology.2014.10.016

Przygrodzka, E., Lopinska, M., & Ziecik, A. J. (2014). Precision-cut luteal slices: A promising approach for studying luteal function in pigs. Reproductive Biology, 14(3), 243-247. doi:10.1016/j.repbio.2014.04.001

Pusateri, A. E., Smith, J. M., Smith, J.,2nd, Thomford, P. J., & Diekman, M. A. (1996). Maternal recognition of pregnancy in swine. I. minimal requirement for exogenous estradiol-17 beta to induce either short or long pseudopregnancy in cycling gilts. Biology of Reproduction, 55(3), 582-589. doi:10.1095/biolreprod55.3.582

Pusateri, A. E., Wilson, M. E., & Diekman, M. A. (1996). Maternal recognition of pregnancy in swine. II. plasma concentrations of progesterone and 13,14-dihydro-15-keto-prostaglandin F2 alpha during the estrous cycle and during short and long pseudopregnancy in gilts. Biology of Reproduction, 55(3), 590-597. doi:10.1095/biolreprod55.3.590

Pusateri, A. E., Rothschild, M. F., Warner, C. M., & Ford, S. P. (1990). Changes in morphology, cell number, cell size and cellular estrogen content of individual littermate pig conceptuses on days 9 to 13 of gestation. Journal of Animal Science, 68(11), 3727- 3735. doi:10.2527/1990.68113727x

Quennell, J. H., Howell, C. S., Roa, J., Augustine, R. A., Grattan, D. R., & Anderson, G. M. (2011). Leptin deficiency and diet-induced obesity reduce hypothalamic kisspeptin expression in mice. Endocrinology, 152(4), 1541-1550. doi:10.1210/en.2010-1100

Quesnel, H., Boulot, S., Serriere, S., Venturi, E., & Martinat-Botté, F. (2010). Post- insemination level of feeding does not influence embryonic survival and growth in highly prolific gilts. Animal Reproduction Science, 120(1), 120-124. doi: 10.1016/j.anireprosci.2010.04.006

Ramirez, A., Karriker, L. A., Johnson, A. K., Colpoys, J. D., Edwards-Callaway, L., Calvo- Lorenzo, M., . . . Radke, S. L. (2019). Diseases of swine, 11th edition, John Wiley and sons, NJ, USA doi:10.1002/9781119350927

Rao, C. V., & Edgerton, L. A. (1984). Dissimilarity of corpora lutea within the same ovaries or those from right and left ovaries of pigs during the oestrous cycle. Journal of Reproduction and Fertility, 70(1), 61-66. doi:10.1530/jrf.0.0700061

Razdan, P., Mwanza, A. M., Kindahl, H., Rodriguez-Martinez, H., Hultén, F., & Einarsson, S. (2002). Effect of repeated ACTH-stimulation on early embryonic development and hormonal profiles in sows. Animal Reproduction Science, 70(1), 127-137. doi:10.1016/S0378-4320(01)00182-8

Redmer, D. A., Dai, Y., Li, J., Charnock-Jones, D. S., Smith, S. K., Reynolds, L. P., & Moor, R. M. (1996). Characterization and expression of vascular endothelial growth factor (VEGF) in the ovine corpus luteum. Journal of Reproduction and Fertility, 108(1), 157- 165. doi:10.1530/jrf.0.1080157

Richards, R. G., Gadsby, J. E., & Almond, G. W. (1994). Differential effects of LH and PGE2 on progesterone secretion by small and large porcine luteal cells. Reproduction, 102(1), 27-34. doi:10.1530/jrf.0.1020027

Roberts, R. M., & Bazer, F. W. (1988). The functions of uterine secretions. Journal of Reproduction and Fertility, 82(2), 875-892. Roberts, R. M., Murray, M. K., Burke, M. G., Ketcham, C. M., & Bazer, F. W. (1987). Hormonal control and function of secretory proteins. Advances in Experimental Medicine and Biology, 230, 137-150

Roberts, R. M., Murray, M. K., Burke, M. G., Ketcham, C. M., & Bazer, F. W. (1987). Hormonal control and function of secretory proteins. Advances in Experimental Medicine and Biology, 230, 137-150

Roberts, R. M., Raub, T. J., & Bazer, F. W. (1986). Role of uteroferrin in transplacental iron transport in the pig. Federation Proceedings, 45(10), 2513-2518

Robertson, H. A., & King, G. J. (1974). Plasma concentrations of progesterone, oestrone, oestradioa-17β and of oestrone sulphate in the pig at implantation, during pregnancy and at parturition. Journal of Reproduction and Fertility, 40(1), 133-141. doi:10.1530/jrf.0.0400133

Sanders, S. L., Stouffer, R. L., & Brannian, J. D. (1996). Androgen production by monkey luteal cell subpopulations at different stages of the menstrual cycle. The Journal of Clinical Endocrinology and Metabolism, 81(2), 591-596. doi:10.1210/jcem.81.2.8636273

Santos, J. E. P., Thatcher, W. W., Chebel, R. C., Cerri, R. L. A., & Galvão, K. N. (2004). The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Animal Reproduction Science, 82-83, 513-535

Senger, P. (2015). Pathways to pregnant and parturition; (3rd ed.) Current Conceptions, Inc.

Shille, V. M., Karlbom, I., Einarsson, S., Larsson, K., Kindahl, H., & Edqvist, L. E. (1979). Concentrations of progesterone and 15-keto-13,14-dihydroprostaglandin F2 alpha in peripheral plasma during the estrous cycle and early pregnancy in gilts. Zentralblatt Fur Veterinarmedizin. Reihe A, 26A(3), 169-181. doi:10.1111/j.1439-0442.1979.tb00663.x

Siawrys, G., & Gajewska, A. (2017). In vitro effect of leptin on anterior pituitary cells LH secretory activity during early pregnancy in pig. Polish Journal of Veterinary Sciences, 20(1), 67-76. doi:10.1515/pjvs-2017-0010

Smith, C. A., & Almond, G. W. (1991). Effects of season and stage of gestation on luteinizing hormone release in gilts. Canadian Journal of Veterinary Research = Revue Canadienne De Recherche Veterinaire, 55(3), 294-297

Smith, J. T., Acohido, B. V., Clifton, D. K., & Steiner, R. A. (2006). KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. Journal of Neuroendocrinology, 18(4), 298- 303. doi:10.1111/j.1365-2826.2006.01417.x

Soede, N. M., Helmond, F. A., & Kemp, B. (1994). Periovulatory profiles of oestradiol, LH and progesterone in relation to oestrus and embryo mortality in multiparous sows using transrectal ultrasonography to detect ovulation. Reproduction, 101(3), 633-641. doi:10.1530/jrf.0.1010633

Soede, N. M., Langendijk, P., & Kemp, B. (2011). Reproductive cycles in pigs. Animal Reproduction Science, 124(3), 251-258. doi://doi.org/10.1016/j.anireprosci.2011.02.025

Soede, N. M., Roelofs, J. B., Verheijen, R., Schouten, W., Hazeleger, W., & Kemp, B. (2007). Effect of repeated stress treatments during the follicular phase and early pregnancy on reproductive performance of gilts. Reproduction in Domestic Animals, 42(2), 135-142. doi:10.1111/j.1439-0531.2006.00742.x

Sonstegard, T. S., Carlson, D., Lancto, C., & Fahrenkrug, S. C. (2016). Precision animal breeding as a sustainable, non-GMO solution for improving animal production and welfare. Paper presented at the Biennial Conference of the Australian Society of Animal Production, 31 316-317

Spies, H. G., Slyter, A. L., & Quadri, S. K. (1967). Regression of corpora lutea in pregnant gilts administered antiovine LH rabbit serum. Journal of Animal Science, 26(4), 768-771

Spoolder, H. A. M., Geudeke, M. J., Van der Peet-Schwering, C. M. C., & Soede, N. M. (2009). Group housing of sows in early pregnancy: A review of success and risk factors. Livestock Science, 125(1), 1-14. doi:10.1016/j.livsci.2009.03.009

Stefanczyk-Krzymowska, S., Wa sowska, B., Chlopek, J., & Grzegorzewski, W. (2004). Retrograde transfer of steroid hormones to the ovary in luteal and follicular phases of porcine oestrous cycle in vivo. Experimental Physiology, 89(1), 140-144. doi:10.1113/expphysiol.2003.002612

Stefanczyk-Krzymowska, S., Grzegorzewski, W., Wasowska, B., Skipor, J., & Krzymowski, T. (1998). Local increase of ovarian steroid hormone concentration in blood supplying the oviduct and uterus during early pregnancy of sows. Theriogenology, 50(7), 1071- 1080

Stone, B. A., & Seamark, R. F. (1985). Steroid hormones in uterine washings and in plasma of gilts between days 9 and 15 after oestrus and between days 9 and 15 after coitus. Reproduction, 75(1), 209-221. doi:10.1530/jrf.0.0750209

Stroband, H. W., & Van der Lende, T. (1990). Embryonic and uterine development during early pregnancy in pigs.Journal of Reproduction and Fertility.Supplement, 40, 261-277

Symonds, H. W., Prime, G. R., & Pullar, R. A. (1994). Preliminary evidence for the enterohepatic circulation of progesterone in the pig. The British Veterinary Journal, 150(6), 585-593

Szafranska, B., & Ziecik, A. (1989). Active and passive immunization against luteinizing hormone in pigs. Acta Physiologica Hungarica, 74(3-4), 253-258

Tast, A., Love, R. J., Clarke, I. J., & Evans, G. (2000). Effects of active and passive gonadotrophin-releasing hormone immunization on recognition and establishment of pregnancy in pigs. Reproduction, Fertility, and Development, 12(5-6), 277-282

Tast, A., Peltoniemi, O. A. T., Virolainen, J. V., & Love, R. J. (2002). Early disruption of pregnancy as a manifestation of seasonal infertility in pigs. Animal Reproduction Science, 74(1-2), 75-86.

Tekpetey, F. R., & Armstrong, D. T. (1991). Steroidogenic response of rat and pig luteal cells to estradiol-17β and catecholestrogens in Vitro1. Biology of Reproduction, 45(3), 498- 505. doi:10.1095/biolreprod45.3.498

Trigg, T. E., Doyle, A. G., Walsh, J. D., & Swangchan-uthai, T. (2006). A review of advances in the use of the GnRH agonist deslorelin in control of reproduction. Theriogenology, 66(6-7), 1507-1512. doi:S0093-691X(06)00111-7

Turner, A. I., Hemsworth, P. H., Canny, B. J., & Tilbrook, A. J. (1999). Sustained but not repeated acute elevation of cortisol impaired the luteinizing hormone surge, estrus, and ovulation in gilts. Biology of Reproduction, 61(3), 614-620. doi:10.1095/biolreprod61.3.614

Tzoupis, H., Nteli, A., Androutsou, M., & Tselios, T. (2019). Gonadotropin releasing hormone and GnRH receptor: Structure, function and drug development. Current Medicinal Chemistry, doi:10.2174/092986732666190712165444

van der Meulen, J., Helmond, F. A., & Oudenaarden, C. P. J. (1988). Effect of flushing of blastocysts on days 10–13 on the life-span of the corpora lutea in the pig. Reproduction, 84(1), 157-162. doi:10.1530/jrf.0.0840157

van Loenen, André C. D., Huirne, J. A. F., Schats, R., Hompes, P. G. A., & Lambalk, C. B. (2002). GnRH agonists, antagonists, and assisted conception. Seminars in Reproductive Medicine, 20(04), 349-364. doi:10.1055/s-2002-36713

Virolainen, J. V., Love, R. J., Tast, A., & Peltoniemi, O. A. (2004). Effect of a gonadotrophin-releasing hormone antagonist on luteinising hormone secretion and early pregnancy in gilts. Reproduction, Fertility, and Development, 15(8), 451-459

Virolainen, J. V., Love, R. J., Tast, A., & Peltoniemi, O. A. (2005). Plasma progesterone concentration depends on sampling site in pigs. Animal Reproduction Science, 86(3-4), 305-316. doi:S0378-4320(04)00198-8

Waclawik, A., Rivero-Muller, A., Blitek, A., Kaczmarek, M. M., Brokken, L. J., Watanabe, K., . . . Ziecik, A. J. (2006). Molecular cloning and spatiotemporal expression of prostaglandin F synthase and microsomal prostaglandin E synthase-1 in porcine endometrium. Endocrinology, 147(1), 210-221. doi:en.2005-0880

Waclawik, A., Kaczmarek, M. M., Kowalczyk, A. E., Bogacki, M., & Ziecik, A. J. (2008). Expression of prostaglandin synthesis pathway enzymes in the porcine corpus luteum during the oestrous cycle and early pregnancy. Theriogenology, 70(2), 145-152

Waclawik, A., Kaczmarek, M. M., Blitek, A., Kaczynski, P., & Ziecik, A. J. (2017). Embryo- maternal dialogue during pregnancy establishment and implantation in the pig. Molecular Reproduction and Development, 84(9), 842-855. doi:10.1002/mrd.22835

Wakabayashi, Y., Nakada, T., Murata, K., Ohkura, S., Mogi, K., Navarro, V. M., . . . Okamura, H. (2010). Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. The Journal of Neuroscience, 30(8), 3124-3132. doi:10.1523/JNEUROSCI.5848-09.2010

Walsh, S. W., Williams, E. J., & Evans, A. C. O. (2011). A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science, 123(3), 127- 138. doi: 10.1016/j.anireprosci.2010.12.001

Walters, D. L., Schams, D., & Schallenberger, E. (1984). Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. Journal of Reproduction and Fertility, 71(2), 479-491. doi:10.1530/jrf.0.0710479

Wasielak, M., Glowacz, M., Kaminska, K., Waclawik, A., & Bogacki, M. (2008). The influence of embryo presence on prostaglandins synthesis and prostaglandin E2 and F2alpha content in corpora lutea during periimplantation period in the pig. Molecular Reproduction and Development, 75(7), 1208-1216. doi:10.1002/mrd.20862

Wasowska, B., & Stefanczyk-Krzymowska, S. (2009). Local effect of progesterone infusion into the porcine ovarian artery on androgen and estrogen secretion during the middle luteal phase. Reproductive Biology, 9(2), 161-179. doi:S1642-431X(12)60024-2

Whisnant, C. S., & Harrell, R. J. (2002). Effect of short-term feed restriction and refeeding on serum concentrations of leptin, luteinizing hormone and insulin in ovariectomized gilts. Domestic Animal Endocrinology, 22(2), 73-80

Wientjes, J., Soede, N. M., van, d. B., & Kemp, B. (2012). Nutritionally induced relationships between insulin levels during the weaning-to-ovulation interval and reproductive characteristics in multiparous sows: II. luteal development, progesterone and conceptus development and Uniformity1. Reproduction in Domestic Animals, 47(1), 62-68. doi:10.1111/j.1439-0531.2011.01802.x

Williams, I. A., Shaw, R. W., & Burford, G. (1989). An attempt to alter the pathophysiology of polycystic ovary syndrome using a gonadotrophin hormone releasing hormone agonist--nafarelin. Clinical Endocrinology, 31(3), 345-353. doi:10.1111/j.1365-2265.1989.tb01258.x

Willis, H. J., Zak, L. J., & Foxcroft, G. R. (2003). Duration of lactation, endocrine and metabolic state, and fertility of primiparous sows. Journal of Animal Science, 81(8), 2088-2102. doi:10.2527/2003.8182088x

Woody, C. O., First, N. L., & Pope, A. L. (1967). Effect of exogenous progesterone on estrous cycle length. Journal of Animal Science, 26(1), 139-141. doi:10.2527/jas1967.261139x

Wu, Y. V., & Burnham, W. M. (2018). Progesterone, 5a-dihydropogesterone and allopregnanolone's effects on seizures: A review of animal and clinical studies. Seizure, 63, 26-36. doi:S1059-1311(18)30569-7

Wuttke, W., Pitzel, L., Seidlová-Wuttke, D., & Hinney, B. (2001). LH pulses and the corpus luteum: The luteal phase deficiency (LPD). Vitamins & Hormones, 63, 131-158. doi:10.1016/S0083-6729(01)63005-X

Wuttke, W., Theiling, K., Hinney, B., & Pitzel, L. (1998). Regulation of steroid production and its function within the corpus luteum. Steroids, 63(5), 299-305. doi:10.1016/S0039-128X(98)00037-3

Ye, R. S., Xi, Q. Y., Qi, Q., Cheng, X., Chen, T., Li, H., . . . Zhang, Y. L. (2013). Differentially expressed miRNAs after GnRH treatment and their potential roles in FSH regulation in porcine anterior pituitary cell. PloS One, 8(2), e57156. doi:10.1371/journal.pone.0057156

Young, K. H., Kraeling, R. R., & Bazer, F. W. (1989). Effects of prolactin on conceptus survival and uterine secretory activity in pigs. Reproduction, 86(2), 713-722. doi:10.1530/jrf.0.0860713

Zhao, Y., Burbach, J. A., Roby, K. F., Terranova, P. F., & Brannian, J. D. (1998). Macrophages are the major source of tumor necrosis factor α in the porcine corpus Luteum1. Biology of Reproduction, 59(6), 1385-1391. doi:10.1095/biolreprod59.6.1385

Zheng, J., Fricke, P. M., Reynolds, L. P., & Redmer, D. A. (1994). Evaluation of growth, cell proliferation, and cell death in bovine corpora lutea throughout the estrous cycle. Biology of Reproduction, 51(4), 623-632. doi:10.1095/biolreprod51.4.623

Zhou, D., Zhuo, Y., Che, L., Lin, Y., Fang, Z., & Wu, D. (2014). Nutrient restriction induces failure of reproductive function and molecular changes in hypothalamus–pituitary– gonadal axis in postpubertal gilts. Molecular Biology Reports, 41(7), 4733-4742. doi:10.1007/s11033-014-3344-x

Zhu, H. S., Qian, Z., Liu, H. L., & Bao, E. D. (2016). ACTH-induced stress in weaned sows impairs LH receptor expression and steroidogenesis capacity in the ovary. Reproductive Biology and Endocrinology, 14(1), 80. doi:10.1186/s12958-016-0214-5

Ziecik, A. J. (2002). Old, new and the newest concepts of inhibition of luteolysis during early pregnancy in pig. Domestic Animal Endocrinology, 23(1-2), 265-275

Ziecik, A. J., Przygrodzka, E., Jalali, B. M., & Kaczmarek, M. M. (2018). Regulation of the porcine corpus luteum during pregnancy. Reproduction, 156(3), R57-R67. doi:10.1530/REP-17-0662

Ziecik, A., Doboszynska, T., & Dusza, L. (1986). Concentrations of LH, prolactin and progesterone in early-pregnant and oestradiol-treated pigs. Animal Reproduction Science, 10(3), 215-224. doi: 10.1016/0378-4320(86)90032-1

Ziecik, A., Shaw, H. J., & Flint, A. P. (1980). Luteal LH receptors during the oestrous cycle and early pregnancy in the pig. Journal of Reproduction and Fertility, 60(1), 129-137. doi:10.1530/jrf.0.0600129

10

Acknowledgements

It takes a village to raise a child… and the same applies to a PhD project. I would like to thank the people who made this dream of mine come true.

My very first employer was Jan-Bernd Lammers. Together with the clients, he showed me the interesting side of porcine veterinary practice. In Finland, years later, I got to know Olli Peltoniemi. He triggered my interest in endocrinology and became my supervisor. His never-ending optimism, deep trust in me and the freedom given were the backup I needed. My second supervisor, Mari Heinonen, was the person to contact for practical advice on the process and people. Both also made their hands dirty in the stable and in the laboratory – thanks for that. Nicoline Soede, my third supervisor, accrued a bit later. She was the most critical reader of my manuscripts and sharply addressed weak points. Through her, I got to know Lia Hoving. Lia's support during the second study and her co-authorship in the third article were highly appreciated.
I should also not forget to mention Eve Ala-Kurikka, who I met in the beginning of my PhD studies. The daily journey to and from Saari with her belong to my dearest memories of my start in Finland. She gave me lots of insight into Finnish veterinary practice and way of thinking. She was part of the pig research group along with Claudio Oliviero and Stefan Björkman and many others. In Viikki there were Satu Sankari, Merja Pöytäkangas, Taina Rahkonen and Saija Vehmas, whose support in the laboratory work was much appreciated.
The help of numerous students and staff during bloodsampling, of Hannes Kauffold who introduced the grey scale ultrasound, of Laura Hänninen who euthanised the sows during my pregnancy and of Viivi Deckwirth and Pernilla Syrjä who organised the cross-section at the pathology institute and of Angelica Hüssler who explained statistics to me was highly valued.
My two pre-examiners Ylva Sjunnesson and Chris Groupen took the time to read my thesis thoroughly. Their advice and positive feedback were very helpful.

Outside university, I was supported by my employers and colleagues in veterinary practice. In particular, I would like to mention Tiina Laitala and Paula Toura in Rauma and in Siilinjärvi it was Kaisa Hartikainen and Laura

Malinen and other colleagues who organised and carried the extra work caused by my absence.

Personally, I would like to thank Finland, as a country that supports working mothers and offers high quality education. I have really come to appreciate that.
During the PhD process, my three children Eliel, Lauren and Neah were born. You were the ones who made me laugh and gave lightheartedness and showed me over and over again what really counts in life. My family is only complete with my husband Willem. I asked a lot from you during starting, continuing and finishing this project. You were there, listened, supported, took care of the children, transported sows, designed figures, even learned pipetting, took my frustrations and gave back love. I am blessed to have you in my life.

11

Original articles

Cover
Neah Haen
Gais, 2020

Layout
Willem Haen
Gais, 2020

Print
Unigrafia Oy
Helsinki, 2020